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CONTENTS OF VOLUME XXIV.

Nos. 1/2.

		PAGES
1.	Willmott, S. Gametogenesis, and Early Development in *Gigantocotyle bathycotyle* (Fischoeder, 1901), Näsmark, 1937	1–14
2.	Hunter, G. C. and Kille, R.A. Some Observations on *Dictyocotyle coeliaca* Nybelin, 1941 (Monogenea) ...	15–22
3.	LeRoux, P. L. *Trichostrongylus leiperi* sp. nov., a Parasite of the Eland (*Taurotragus oryx*) in Northern Rhodesia	23–27
4.	LeRoux, P. L. A Trichostrongylid, *Paracooperia mazabukae* sp. nov., from a wild ruminant, the Oribi, in Northern Rhodesia	28–32
5.	Madsen, H. On the Systematics of *Syngamus trachea* (Montagu, 1811) Chapin, 1925	33–46
6.	Whitlock, H. V. A Technique for Counting Trematode Eggs in Sheep Faeces	47–52
7.	Clapham, P. A. On Treating and Preventing Gapeworm Disease	53–60
8.	Clapham, P. A. Keratitis in Pheasants following Treatment with Phenothiazine	61–62
9.	Kendall, S. B. Snail Hosts of *Fasciola hepatica* in Britain...	63–74
10.	Fenwick, D. W. Investigations on the Emergence of Larvae from Cysts of the Potato-Root Eelworm *Heterodera rostochiensis*. 2. The Form of the Hatching Curve	75–86
11.	Fenwick, D. W. Investigations on the Emergence of Larvae from Cysts of the Potato-Root Eelworm *Heterodera rostochiensis*. 3. Larval Emergence in soil under the influence of Potato-Root Diffusate	86–90
12.	Staniland, L. N. Notes on the use of Iodine and Chlorphenol against certain nematodes	91–99
	Actual Dates of Publication, Volume XXIII	100

No. 3.

PAGES

1. Morgan, D. O., Parnell, I. W. and Rayski, C. Further Observations on the Seasonal Variation in Worm Egg Output in Scottish Hill Sheep 101–122

2. Edwards, E. E. Experiments with D-D Mixture Against Root-Knot Eelworm, *Heterodera marioni* (Cornu)... 123–136

3. Clapham, P. A. On Sterilizing Land Against Poultry Parasites 137–144

4. Southey, J. F. and Staniland, L. N. Observations and Experiments on Stem Eelworm, *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936, with Special Reference to Weed Hosts 145–154

No. 4.

1. Willmott, S. On the species of *Paramphistomum* Fischoeder, 1901 Occurring in Britain and Ireland with Notes on Some Material from the Netherlands and France 155–170

2. Sandosham, A. A. On *Enterobius vermicularis* (Linnaeus, 1758) and Some Related Species from Primates and Rodents 171–204

Index 205–210

Journal of Helminthology, Vol. XXIV, *Nos.* 1/2, 1950, *pp.* 1—14.

*Gametogenesis, and Early Development in *Gigantocotyle bathycotyle* (Fischoeder, 1901) Näsmark, 1937.

By S. Willmott, B.Sc., Ph.D.

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The cytology and germ cell cycles of digenetic trematodes are of particular interest in view of their complicated life histories, their hermaphroditism and the difficulties in taxonomy of these parasites.

There are numerous different opinions on synonomy in the family Paramphistomidae as expressed by Fischoeder (1901, etc.), Maplestone (1923), Stunkard (1925), Fukui (1929), Travassos (1934), Dawes (1936), and Näsmark (1937). Most of these authors have different views as to what constitutes a character of generic or specific importance.

It is hoped that further light will be shed on the question by means of detailed studies on the chromosomes and germ cell cycles in a number of closely related members of the family. This paper deals with the processes in *Gigantocotyle bathycotyle* (Fischoeder, 1901), Näsmark, 1937.

Some difficulty was experienced in identifying the material. After study of a large number of slides in the collection of the London School of Hygiene and Tropical Medicine, it appears that the classification proposed by Näsmark in 1937 is reliable and the most easily workable. For the purposes of the present paper it is assumed that *Gigantocotyle bathycotyle* is not synonymous with *Gigantocotyle explanatum.*

As many of the earlier papers which give an account of gametogenesis appear to be based on different interpretations of nuclear division, it is proposed to give a definition of terms used in this paper. These are based on those given by White (1946).

Leptotene; corresponds to the earliest part of mitotic prophase. The chromosomes are very long slender threads with numerous chromomeres distributed along their length. According to White, Darlington believes that the chromosomes are unsplit at this time, and that this constitutes a distinction of primary importance between leptotene and the corresponding stage of somatic mitosis.

Zygotene; the homologous chromosomes come together, side by side, throughout their entire length.

Pachytene; the pairing process is complete. The appearance of the chomosome threads resembles that of mid-prophase chromosomes at mitosis.

* Part of a thesis approved by the University of London for the award of the Ph.D. Degree.

Diplotene; the attraction between the homologous chromosomes seems to end and the pairs separate, remaining held together by chiasmata.

Diakinesis; corresponds to late prophase of somatic mitosis.

Bivalent; two homologous chromosomes which have completed the process of pairing and appear as one body.

Material and Methods.

The material was collected from the rumen of *Bos indicus* at the Colombo Municipal Slaughter House, Ceylon. It was fixed in Carnoy (6 : 3 : 1) and preserved in 90% alcohol. Material for sectioning was embedded in paraffin wax with ceresin, congealing point 52° C. or 54° C., as it was found that after embedding in a wax with a higher congealing point, the material became very brittle. Sections for the purpose of identification and study of general anatomy were cut at 20μ and stained in Borax-carmine or Ehrlich's Haematoxylin, with Eosin as counter-stain.

For demonstration of spermatogenesis, sections were cut 4–6μ in thickness and stained in Weigert's Iron Haematoxylin or Heidenhain's Iron Haematoxylin without counter-stain. Most satisfactory results were obtained with Heidenhain's Iron Haematoxylin, using the following method. Mordant sections for two hours in 5% iron alum solution in 50% alcohol. Stain for 12–15 hours in 1% haematoxylin, differentiate with a saturated solution of picric acid in 70% alcohol. This differentiation is very rapid and should be watched under a binocular microscope.

For demonstration of oogenesis and early development, sections were cut 4–12μ in thickness and stained with Weigert's Iron Haematoxylin, Heidenhain's Iron Haematoxylin, without counter-stain, and Ehrlich's Haematoxylin counter-stained with eosin.

The Feulgen staining reaction was tried with little success. According to Britt (1947) it is more satisfactory if material is fixed for five minutes in Carnoy, followed by two hours in San Felice. When more fresh material is available, it is hoped to try this technique.

Except where otherwise stated, all drawings were made with the aid of a camera lucida.

Anatomy of the Genitalia.

Male.

There are two testes lying one behind the other, the posterior tending to be wedge-shaped. Both are very slightly lobed and smooth in outline. A vas deferens runs anteriorly from each testis joining to

form a long, thin-walled vesicula seminalis. In mature worms this is packed with spermatozoa and much coiled. The vesicula seminalis leads into a pars musculosa which is not very strongly developed and this in turn passes into a pars prostatica. The cells surrounding this part of the duct agree very closely histologically with those of Mehlis gland. From the pars prostatica the ductus ejaculatorius opens into the genital atrium.

The following are measurements taken from thick hand sections. All the worms are mature and there is very little variation in size.

	Anterior testis	Posterior testis
Length (*anterior-posterior*) ...	1.5 mm.	1 mm.
Depth (*dorsal-ventral*)	2.5 mm.	2.5 mm.
Breadth (*side-side*)	2.5 mm.	3.5 mm.

Female. (Figs. 1, 2, 3.)

The single ovary lies posterior to the testes and dorsal to the acetabulum. It is pear-shaped and measures about 0·4 mm. × 1 mm. × 1 mm. Oogonia form a cap opposite to the opening of the oviduct and occupy about one-third of the ovary (Fig. 3).

The oviduct is a slightly coiled narrow tube running posteriorly, dorsal to Mehlis gland. It is joined by Laurer's canal outside Mehlis gland, thus differing from *Fasciola hepatica*, in which the two ducts join within the gland (Stephenson, 1947). Laurer's canal opens to the exterior on the dorsal surface, in the mid line, about the level of the ovary (Fig. 2). No spermatozoa nor surplus vitelline material have been found in Laurer's canal. The oviduct runs into Mehlis gland, where it is joined by the vitelline duct and forms the central chamber of the gland. The diameter of this is very variable according to the number of vitelline cells which it contains. No valve such as that described by Stephenson in *F. hepatica* has been observed.

The uterus leads from the central chamber and passes anteriorly. In the most proximal part of the uterus the eggs lie singly, but become closely packed in the more distal portions.

There is neither a receptaculum seminis nor a receptaculum uterinum, but in all the specimens sectioned there appears to be one region of the uterus into which spermatozoa are concentrated. This loop lies between the posterior border of the testis, and the ovary. It was at first thought to be a receptaculum uterinum but further observations showed it to be continuous with the uterus and, in some specimens, to contain eggs. When packed with eggs the uterus becomes much convoluted and extends over almost the whole dorsal surface of the worm.

The metraterm, or vagina, is not clearly differentiated from the rest of the uterus and does not appear to be very muscular. It opens into the genital atrium just below the male opening.

Mehlis gland is compact and more or less spherical. The region of intracellular ducts is not as extensive as in *F. hepatica* (Stephenson, 1947).

The vitellaria are follicular and extend from the level of the pharynx to the middle of the acetabulum. A vitelline duct runs in dorsally to the acetabulum from each side and these join to form a vitelline reservoir adjacent to Mehlis gland. From this a single duct leads into the central chamber of the gland.

Gametogenesis.

Spermatogenesis (Figs. 4–26).

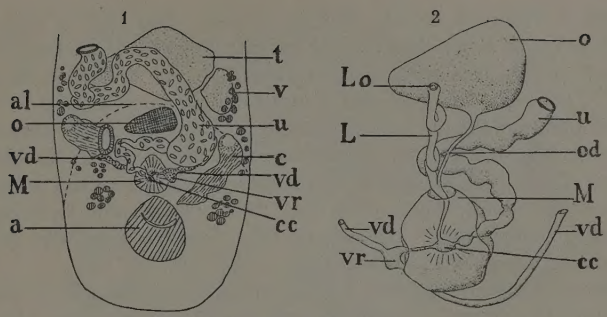
The testes are bordered by a layer of fibrous tissue which appears to be derived from the cells of the parenchyma. Within this is a layer, from one to six cells in thickness, of primordial spermatogonia ; in mature worms the usual thickness is two or three cells.

The nuclei of these cells are usually in the resting stage and there seems to be no distinct karyosome (Fig. 4*a*). Division takes place rapidly and is a process of normal mitosis. As the cells are very closely packed and the nuclei vary considerably in size, measurement is difficult. During prophase nucleination of the chromosomes begins (Fig. 4*b*). They become visible as double threads and in some cases it is possible to observe the pairs of chromomeres along the whole length of the chromosome (Fig. 4*c*). It is unknown at what stage the longitudinal splitting of each chromosome into two chromatids takes place but as they appear as double threads, it is before prophase. It may be during the resting stage after the previous division (Darlington, 1935) or earlier, according to some authors, as given by White (1946).

As nucleination and spiralisation continue the chromomeres become invisible and by the time metaphase is reached the chromosomes

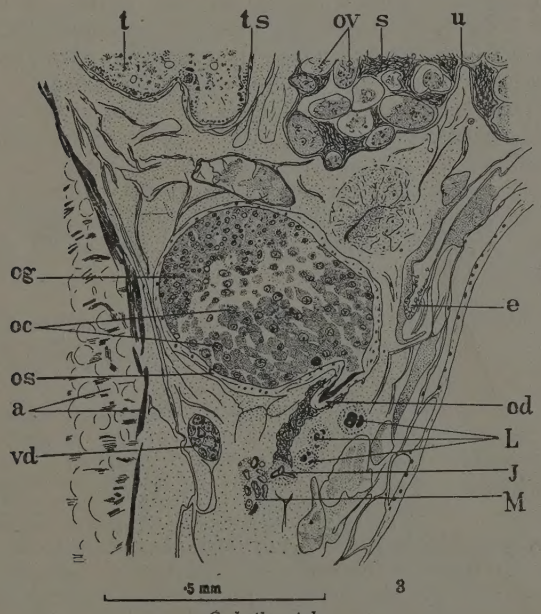
Abbreviations used in Figures 1–38.

a—acetabulum ; **al**—anterior limit of acetabulum ; **c**—gut caecum ; **cc**—central chamber of Mehlis gland ; **e**—excretory bladder ; **f**—fertilisation membrane ; **j**—junction of oviduct with Laurer's canal ; **L**—Laurer's canal ; **Lo**—opening of Laurer's canal to exterior ; **M**—Mehlis gland ; **o**—ovary ; **oc**—oocyte ; **od**—oviduct ; **og**—oogonia ; **os**—fibrous layer surrounding ovary ; **ov**—eggs in uterus ; **p**—pronucleus ; **pbl**—first polar body ; **pr**—propagatory cell ; **s**—spermatozoa in uterus ; **sc**—spermatozoon in cytoplasm of oocyte ; **sh**—shell ; **t**—testis ; **ts**—fibrous layer surrounding testis ; **u**—uterus ; **v**—vitelline glands ; **vc**—vitelline cells ; **vd**—vitelline duct ; **vr**—vitelline reservoir ; **w**—wall of central chamber of Mehlis gland.



Genitalia of *G. bathycotyle.*

Fig. 1. Diagrammatic representation of thick (hand) horizontal section. Oviduct and Laurer's canal omitted. Not to scale. Fig. 2. Ovary, Mehlis gland and associated ducts reconstructed from serial sections. Dorsal view. Scale only approximate.



G. bathycotyle.

Fig. 3. Sagittal section through ovary to show the junction of the oviduct and Laurer's canal.

appear as compact densely staining bodies. The nuclear membrane disappears and a spindle is formed ; neither astral rays nor centrosomes have been observed with certainty but this may be due to the small size of the cells. In one case a small darkly stained body was seen which was thought to be a centrosome. At metaphase the chromatids separate (Fig 4, *d* and *e*). No centromeres are visible in the chromosomes. During anaphase (Fig. 4*f*) the chromatids move apart to opposite poles of the spindle where they enter into telophase and two daughter nuclei are reorganised. These pass into a resting stage and grow to the size of the parent cell (Fig. 4*g*).

After an unknown number of mitotic divisions the cells move from the layer of primordial spermatogonia into the testis proper where the three spermatogonial divisions take place. There does not seem to be any zoning of the various stages in spermatogenesis as described by Pin Dji Chen (1937) in *Paragonimus kellicotti*. All stages from primary spermatogonia to free spermatozoa were found throughout the testis and in many cases could be seen in the same section. It is possible that this is due to the degree of maturity of the worms. Cable (1931) working on *Cryptocotyle lingua* found that in mature worms the primary spermatogonia were located near the edge of the gonad.

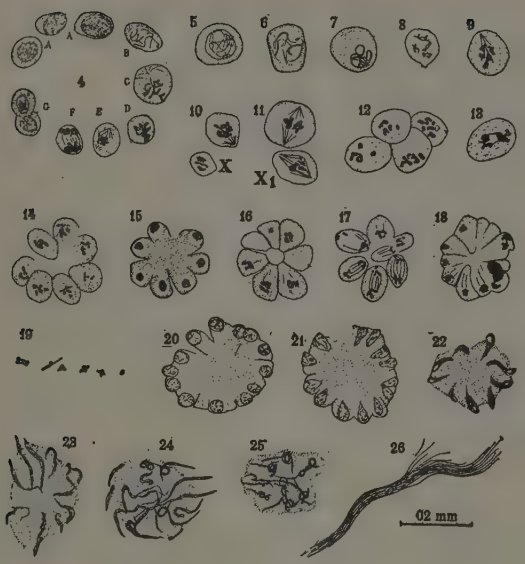
The three spermatogonial divisions take place in the same plane and the daughter cells remain together. This results in a plate of eight cells which are the primary spermatocytes. It is at this stage that both nuclei and cells increase considerably in size. Although measurement of cells, other than primary spermatocytes was difficult, and not very accurate, the following table gives an idea of the amount of growth which takes place.

DIMENSIONS OF CELLS IN PERIPHERAL LAYER OF THE TESTIS.

	Primordial and primary spermatogonia.		Primary spermatocytes.	
	Diameter of Cell	*Diameter of Nucleus*	*Diameter of Cell*	*Diameter of Nucleus*
Minimum	6·4μ	4·8μ	8·0μ	6·4μ
Maximum	9·6μ	7·2μ	11·2μ	8·8μ
Average	7·7μ	5·7μ	9·6μ	7·3μ

No reduction in the number of chromosomes has so far taken place.

Sufficient stages of the reduction division have been observed to indicate that it is a normal meiosis. During leptotene the chromosomes become visible as long thin threads (Fig. 5). They do not appear to be double but as the chromomeres are closer together and not as distinct



Spermatogenesis in *G. bathycotyle*.

Fig. 4. Stages of mitosis in cells of the peripheral layer from various sections. *a*. Resting nuclei. *b*. Early prophase. *c*. Late prophase. *d*. Metaphase—polar view. *e*. Metaphase—side view. *f*. Anaphase. *g*. Two daughter nuclei in resting stage. Fig. 5. Leptotene. Fig. 6. Zygotene. Fig. 7. Pachytene. Fig. 8. Diplotene. Fig. 9. Diakinesis. Figs. 10 and 11. First metaphase. Two sections through the same group of primary spermatocytes. X, XI the same cell. Fig. 12. Polar view of four primary spermatocytes in first metaphase. Fig. 13. Early first anaphase. Two bivalents still remain connected and are stretched on the spindle. Fig. 14. Late first anaphase. Seven out of sixteen secondary spermatocytes are shown. Fig. 15. Interphase. Fig. 16. Polar view of second metaphase. Seven out of sixteen cells shown. Fig. 17. Second anaphase. Fig. 18. End of second maturation division. Chromosomes are still distinct. Fig. 19. Chromosomes from the cell in Fig. 9. Fig. 20. Twelve out of thirty-two spermatid nuclei in the resting stage after the second maturation division. Fig. 21. Spermatid nucleus becoming ovoid and pushing out cell wall. Fig. 22. Spermatid nucleus still more elongated and becoming more densely staining. The nuclei appear to protrude through cell walls. Fig. 23. Tail of spermatozoon being formed from spermatid nucleus. Chromosomes are still visible as twisted threads. Fig. 24. Spermatozoa beginning to coil within the cytoplasmic mass. Fig. 25. Spermatozoa tightly coiled, still lying on cytoplasmic mass. Fig. 26. Bunch of free spermatozoa lying in the testis.

as in a corresponding stage of mitosis, it is impossible to be certain. Zygotene has not been observed very frequently so that it seems likely that the actual pairing of homologous chromosomes takes place extremely rapidly (Fig. 6).

At pachytene (Fig. 7), which seems to last a relatively long time, the bivalents become arranged as loops with all their free ends together at one side of the nucleus. According to White (1946) this is a genuine phenomenon and not a fixation artefact. The chromosomes still retain a slightly woolly appearance which is not lost completely until the end of diakinesis. Condensation continues—during diplotene the pairs open out slightly, remaining held together only by chiasmata and lose their looped appearance (Fig. 8).

The pairs spread out through the nucleus at diakinesis (Fig. 9). The nuclear membrane disappears and a spindle is formed. In a few nuclei centrosomes are distinguishable, but they are extremely small and it is impossible to see if the centrioles have divided. No astral rays have been observed.

The chromosomes are now at their maximum density and lie on the equatorial plate of the spindle in a typical metaphase arrangement (Figs. 10, 11, 12). At this stage the bivalents are widely separated from each other and it is comparatively easy to count them. There is no evidence of any chromosome remaining unpaired and forming a univalent. The homologous pairs separate and one chromosome from every pair passes to each pole (Figs. 13, 14).

The interphase between the first and second division is extremely short (Fig. 15). As soon as anaphase is completed denucleination of the chromosomes proceeds until they resemble those in early prophase of a somatic mitosis. These then undergo a normal mitotic division which results in the formation of thirty-two nuclei, with the haploid number of chromosomes (Figs. 16, 17, 18). Nuclear membranes are reformed but the cytoplasm remains incompletely divided so that the spermatids do not separate but remain in a rosette (Fig. 20).

Spermatozoa are formed from the spermatids without further division. After a short resting stage, during which the chromosomes do not become completely invisible, the spermatid nucleus begins to elongate (Fig. 21). The side towards the periphery of the cytoplasmic mass becomes pointed and pushes up against the cell wall. It then appears to protrude through the wall, but whether the wall is actually ruptured, or stretches to form a thin membrane surrounding the "head" of the future spermatozoon, it is impossible to say (Figs. 22 and 23).

The nucleus continues to elongate and to take stain more densely, and the individual chromosomes become indistinguishable. As the nucleus becomes longer and thinner, it coils within the cytoplasm. Finally the spermatozoa uncoil, free themselves from the cytoplasm and pass into the testis where they lie in bundles, gradually separating and entering the vas deferens (Fig. 26).

The spermatozoa are long and threadlike, with a small indistinct "head." This is more apparent in spermatozoa found in the uterus than in those lying in the testis and vesicula seminalis.

It seems that the whole spermatozoon is derived from nuclear material but it is not impossible that a small amount of cytoplasm is involved. (This seems unlikely, however, in view of the fact that the whole spermatozoon penetrates the oocyte prior to fertilisation.) A similar state of affairs is described by Cable (1931) in *Cryptocotyle lingua* ; Anderson (1937) in *Proterometra macrostoma* ; Rees (1939) in *Parorchis acanthus* ; and Markell (1943) in *Probilotrema californiense*. Chen (1937) is uncertain if this is the case in *Paragonimus kellicotti* and Woodhead (1931) working on the Bucephalidae states that the cytoplasm forms the tail of the spermatozoon.

Oogenesis. (Figs. 27–35.)

Oogonia and primary oocytes only are found in the ovary (Figs. 27, 28). The nuclei of the oocytes differ from those of the spermatocytes in that they contain a distinct karyosome. This is usually spherical but does not appear to be homogeneous. The peripheral part stains very deeply and within this there appear to be two or three bodies which do not stain so intensely. The oogonia and oocytes are larger than the corresponding stages in spermatogenesis ; oogonial divisions are normal mitoses.

The primary oocytes pass singly into the oviduct where it is assumed that they are penetrated by a spermatozoon although this has not been observed. The oocytes travel down the oviduct to the central chamber of Mehlis gland where they become surrounded by vitelline cells. The vitelline cells then give up the drops of shell-forming substances from their cytoplasm and these drops pass to the outside of the group of cells where they coalesce to form the shell (Fig. 29). At this stage the shell is very plastic. A few vitelline cells may remain outside the egg. Within the egg the cytoplasm of the vitelline cells breaks down but the nuclei persist for a considerable time.

In the most proximal part of the uterus a fertilisation membrane appears around the oocyte and the long threadlike spermatozoon can be seen within the cytoplasm (Figs. 30, 31). It seems probable that if

the oocyte is penetrated by a spermatozoon in the oviduct the fertilisation membrane would be formed there. As, however, it is not apparent until the egg is in the uterus it is possible that spermatozoa are enclosed within the shell and that penetration does not take place until later. This membrane only persists for a short time and disappears by the time the spindle of the first maturation division is formed (Fig. 32).

The primary oocyte nucleus remains unchanged until the egg has passed into the uterus. The spermatozoon within the cytoplasm becomes shorter and broader but remains a densely staining compact body for some time. When the spermatozoon has reached this stage the first division of the oocyte nucleus takes place very rapidly. None of the early stages of prophase have been observed, although a few nuclei were found in metaphase. A spindle is formed and six bivalents, resembling those of primary spermatocytes, appear on the equatorial plate. Anaphase follows and the first polar body is extruded ; this may, or may not, divide again, but in one instance was observed in anaphase. There is no interphase, the second division following immediately and a second polar body is given off (Fig. 33).

While these divisions are taking place, the spermatozoon rounds up to form the male pronucleus and chromosomal threads become distinguishable. The chromosomes of the secondary oocyte pass into a resting stage and a nuclear membrane is formed. This is the female pronucleus. Both pronuclei possess a single karyosome and are indistinguishable from one another (Fig. 34).

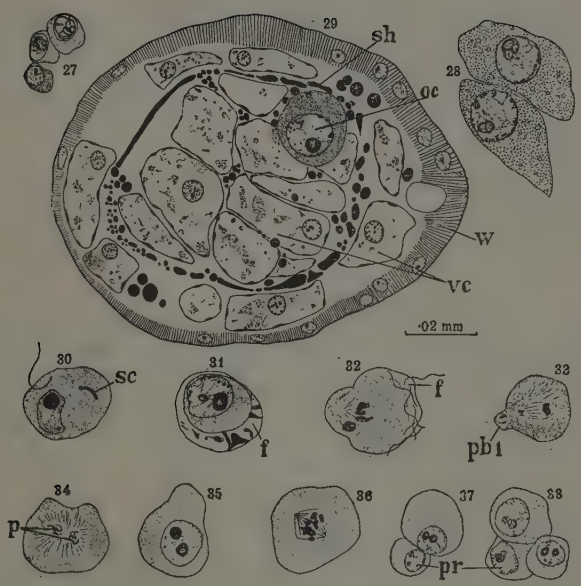
Fusion of the pronuclei has not been observed but a number of cells show a single large nucleus in a resting condition, which contains two karyosomes, and it is assumed that this is a fusion nucleus (Fig. 35).

Tanning of the egg shell takes place gradually along the whole length of the uterus. When first formed, the shell is not birefringent but becomes increasingly so as it travels up the uterus.

Chromosomes (Fig. 19).

It is not possible to make accurate counts or descriptions of the chromosomes at any stage before diakinesis and metaphase of meiosis. At this stage six bivalents are distinguishable ; it is therefore concluded that the diploid number of chromosomes for this species is twelve, the complement being made up of eight short and four long chromosomes. It is difficult to distinguish individual chromosomes.

The only other member of the family Paramphistomidae which has been studied cytologically is *Diplodiscus temporatus*. In this form the chromosome number is given as sixteen (Cary, 1909).



Oogenesis in *G. bathycotyle*.

Fig. 27. Oogonia in ovary. Fig. 28. Primary oocytes in ovary. Fig. 29. Shell formation. Oocyte and vitelline cells in the central chamber of Mehlis gland. Fig. 30. Primary oocyte—vitelline cells and shell omitted—with spermatozoon in cytoplasm. A spermatozoon which has failed to penetrate can be seen lying on the surface of the oocyte. The karyosome of the oocyte nucleus appears denser than in unpenetrated cells and the cytoplasm becomes more finely granular in appearance. The fertilisation membrane has not yet been formed. Fig. 31. Oocyte with fertilisation membrane. Fig. 32. First metaphase of the primary oocyte with three bivalents on the spindle and the spermatozoon becoming rounded. Fig. 33. Second maturation division, with the nucleus of the first polar body in anaphase. Fig. 34. Organisation of the male and female pronuclei.

Cleavage in *G. bathycotyle*.

Fig. 35. Fertilised ovum with fusion nucleus. Fig. 36. First cleavage division. Metaphase. Fig. 37. Two celled stage. Fig. 38. Three celled stage.

Cleavage (Figs. 36–38).

The first cleavage division is a normal mitosis and gives rise to two cells of unequal size (Fig. 36). These probably correspond to the "ectodermal" and "propagatory" cells described by Ishii (1934) in the development of *Fasciolopsis buski*. Similar cells have been reported in *Paragonimus kellicotti* by Pin Dji Chen (1937) and in *Parorchis acanthus* by Rees (1939). The larger of the two cells then divides again, the "propagatory" cell remaining unchanged (Figs. 37, 38).

Owing to the difficulty in getting fixatives to penetrate the egg shell and the extreme brittleness of the shell which results in tearing and distortion in the sections, it has not been possible to follow cleavage any further.

Discussion.

The number of papers on gametogenesis in trematodes is not large. The earlier work has been admirably reviewed by Brooks (1930), since when descriptions of this process have been published by Cable (1931) in *Cryptocotyle lingua*; Woodhead (1931) in the family Bucephalidae; Pennypacker (1936 and 1940) in *Pneumonoeces medioplexus* and *P. similiplexus*; Anderson (1937) in *Proterometra macrostoma*; Chen (1937) in *Paragonimus kellicotti*; Rees (1939) in *Parorchis acanthus*; and Markell (1943) in *Probilotrema californiense*.

There are a number of differences in these accounts which seem to have arisen from three main causes, namely, a lack of definition of terms, variations in appearances caused by the use of different fixatives and different interpretations based on early literature on cytology. The only account which seems to contain really fundamental differences is that given by Woodhead (1931) in the Bucephalidae.

It seems unlikely in the light of recent cytological studies that the chromosomes are, at any stage, in the form of a continuous spireme as described by Woodhead, Chen and Anderson. They certainly do not appear to be in *Gigantocotyle bathycotyle*. Cable states that the filament may be continuous, but that such continuity has not been traced. He also notes the double appearance of the threads before loop formation (pachytene) showing that the homologous chromosomes have undergone pairing.

The account of gametogenesis in the Bucephalidae given by Woodhead is not very clear but the following differences from other accounts are apparent:

(*a*) Groups of spermatogonia fuse before their nuclei undergo reduction division.

(*b*) The nuclei of the spermatocytes become smaller before division takes place.

(*c*) The cytoplasm forms the tail of the spermatozoon.

Woodhead does not say if the whole of the spermatozoon penetrates the oocyte.

SUMMARY.

1. The anatomy of the genitalia of *Gigantocotyle bathycotyle* is described.

2. An account is given of gametogenesis, egg-shell formation, and the first two cleavage divisions.

3. The chromosome number is given as n = 6, 2n = 12.

ACKNOWLEDGMENTS.

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Some Observations on *Dictyocotyle coeliaca* Nybelin, 1941 (Monogenea).

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Dictyocotyle coeliaca Nybelin, 1941, is a trematode dwelling in the coelom of *Raia* spp. This trematode was first described by Nybelin (1941) from two specimens, one obtained from *Raia lintea* Fries, and one from *Raia radiata* Donovan. Later Dawes (1948) gave a brief description from two specimens believed to have been obtained from *Raia clavata* L, and referred the trematode to the genus *Calicotyle* Diesing, 1850, re-naming it *Calicotyle coeliaca* (Nybelin, 1941).

Heavy infestations of the species were observed in rays brought into the Department of Zoology, Edinburgh University, during 1949 from Scottish fishing grounds. On these are based the following account of the distribution of the parasite, and descriptions of the egg and the young forms, which have not previously been described. Notes have been added on the adult and its systematic position.

Distribution.

Table I gives the species of skates and rays examined and the number infested.

Table I.

Species	*Number examined*	*Number infested with* D. coeliaca
R. naevus Muller and Henle	55	9♂ 7♀
R. radiata Donovan	73	13♂ 18♀
R. batis L	7	none
R. montagui Fowler	18	,,
R. brachyura Lafont	2	,,
R. clavata L	23	,,
R. fullonica L	3	,,

* Now at the Rowett Research Institute, Aberdeen.

The number of *D. coeliaca* present in each infested ray varied from 1 to 72, the average being approximately 7. The skates and rays were caught during May and June, 1949, near the Butt of Lewis, the Shetland Isles, and the Coral Bank, E.N.E. of Aberdeen, and infested rays were found in batches from all three areas. In view of these observations regarding the numbers and distribution of the parasite, it is difficult to explain why only four specimens have been previously recorded. It is possible that the wrong species of *Raia* may have been examined, or that poorly preserved specimens have been mistaken for damaged specimens of *Calicotyle kröyeri* Diesing.

The majority of the flukes were without eggs ; a few, however, had a single egg present in the ootype. The eggs (Fig. 2) are 0.215 mm. long (excluding filament) by 0.1 mm. wide, roughly spindle shaped, and triangular in cross section, with a short filament, 0.05 mm. long, at one end. The measurements are those of eggs which were free in the coelom of the host. The filament terminates in a minute knob. At the other end there is an operculum, which is very difficult to see when the eggs are fresh, and which is not quite terminal ; it is formed by the tip of only one of the three sides of the egg.

Fluke eggs were numerous in the coelom and throughout the length of the oviducts of the female host. They were also found inside two egg capsules of one of the specimens of *R. radiata*. In the male host large brown masses ($\frac{1}{2}$ in.–1 in. long) of eggs were found near the anterior end of the coelom. Such masses were not present in the female host and this may be due to the fact that the eggs can more readily escape down the oviducts, the only means of exit in the male host being through the abdominal pores. That such escape is possible may be demonstrated by gently pressing the ventral abdominal wall of a fresh intact ray, when fluke eggs may be seen in the fluid flowing out of the abdominal pores. However, the large numbers of eggs present in the coelom of the male suggests that escape through the abdominal pores is not very efficient and that the female host is mainly responsible for the transmission of the parasite. The eggs in the coelom and the oviducts showed no signs of cleavage.

Eggs which appeared to be those of *D. coeliaca* were also found in the spiral valve of some of the rays. They did not show any signs of development and it is not clear how they reached that position unless they had been ingested by the host.

Immature Forms. Immature as well as adult forms of *D. coeliaca* were found in the coelom. The smallest specimen found (Specimen A, Fig. 1) is 0.57 mm. long (excluding the opisthaptor) by 0.32 mm. at its

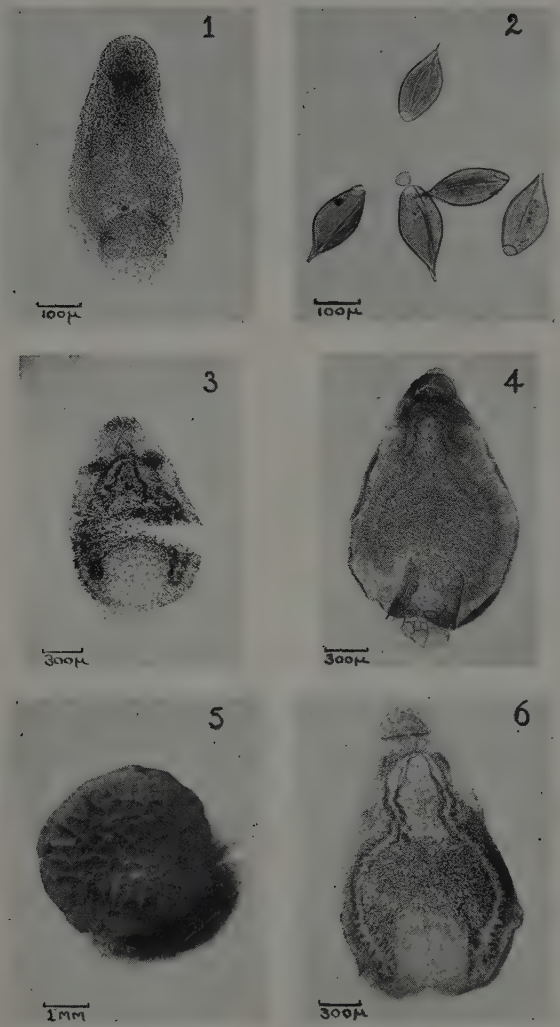

Fig. 1. Specimen A. 0.57 mm. long* × 0.32 mm. wide. Fig. 2. Eggs of *D. coeliaca* showing the operculum. Fig. 3. Specimen B 1.4 mm. long,* × 0.93 mm. wide. Fig. 4. Specimen C. 2.2 mm. long,* × 1.5 mm. wide. Fig. 5. An isolated opisthaptor of an adult showing the arrangement of the loculi. Fig. 6. Specimen D. 2.5 mm. long,* × 1.7 mm. wide.

* excluding opisthaptor.

To face p. 16.

greatest width, and has an opisthaptor 0.2 mm. in diameter. In outline it resembles an elongated pear and is flattened. The opisthaptor projects prominently beyond the posterior margin of the body, as in adults of *C. kröyeri* and other members of the genus *Calycotyle*. If the specimen is overstained and viewed by reflected light before clearing, the opisthaptor can be seen to bear a number of very small shallow loculi. There are no hooks. No internal organs can be recognised except for a slight differentiation of cells giving a vague outline of the gut.

In larger specimens (Spec. B.C. and D., Fig. 3, 4 & 6) the pear shape is not so elongated and the length/breadth ratio gradually decreases up to the adult stage. The loculi of the opisthaptor are more marked than in the smallest specimen, though they are still very shallow. There are about 50 loculi and they are arranged in a pattern similar to those of adults described below. The extent to which the opisthaptor projects beyond the posterior margin of the body gradually decreases with increase in size of the fluke until it is hardly noticeable in the adult. The oral sucker is conspicuous and it appears relatively stronger in the young stage than in the adult. The gut is clearly marked in Specimen B (1.4 mm. long by 0.93 mm. wide) and in larger specimens. At this stage (Specimens B, C and D) the rudiments of the genitalia are visible. Specimens larger than Specimen D (2.5 mm. by 1.7 mm.) have all the adult organs present and only increase in size occurs thereafter. After stage D is reached the anterior end of the body (except the head) widens relatively to the posterior, giving the more oval shape of the adult with a fairly well marked-off head, and a less conspicuous oral sucker.

Adults. The adults have been described by Nybelin (1941) and Dawes (1948), but the following additional notes and a line drawing (Fig. 7) may be of value. The fresh specimens are almost transparent, and often very difficult to see in situ, although sometimes the vitellaria have a bluish tinge. They are usually found at the anterior end of the coelom attached to the wall or to the liver ; less often they are found at the posterior end near the entrance of the abdominal pores.

The largest specimens obtained measure 13 mm. long and the average size of a full grown adult is 11 mm. long by 9 mm. at its greatest width, with an opisthaptor 3.6 mm. in diameter. The opisthaptor (Fig. 5) is flattened and discoidal, and has a broad attachment to the ventral surface of the body. It does not project beyond the posterior margin of the body and has no hooks. On its ventral surface there is

an average number of 60 (varying from 50 to 70) shallow loculi divided by septa. The loculi are irregular in shape and variable in size, but they are arranged in a constant pattern with a peripheral set about twice the size of the more central ones.

The testicular mass extends from behind the ovary to the opisthaptor and fills the space between the caeca. Judging from serial sections, it seems that the mass represents a single testis with many lobes, which diffuse into one another and give the reticulated appearance seen in whole preparations. Vasa efferentia cannot be seen joining to form the vas deferens in serial sections or whole mounts, as would be expected if numerous testes were present. The vas deferens, however, can be seen arising directly from the centre of the anterior border of the testicular mass. The rest of the male genitalia have been described by Nybelin (1941).

The elongated ovary has previously been described by Nybelin (1941) as ending in six spherical terminations and by Dawes (1948) as ending in five such terminations. All the specimens examined here have six spherical terminations except one which has seven.

Systematic Position.

Nybelin (1941) erected a new genus *Dictyocotyle* for the trematode under discussion. Dawes (1948), however, referred the species to the genus *Calicotyle* Diesing, 1850. From a study of the morphological characters seen in many whole preparations and serial sections, a very close relationship appears to exist between *Dictyocotyle coeliaca* and members of the genus *Calicotyle*. There are, however, the following differences.

1. The external openings of the paired vaginae are situated in front of the anterior edge of the vitellaria and lateral to the caeca in *Dictyocotyle coeliaca*. This position is slightly anterior to the level at which they are found in *C. inermis* Woolcock, and well in front of the level found in *C. krōyeri* Diesing.

2. The ovary in *Calicotyle* spp. ends in a simple slight swelling, as in *C. krōyeri*, according to Wierzejski (1877), or in a slightly lobed swelling, as in *C. affinis* Scott, according to Brinkmann (1940). In *D. coeliaca*, on the other hand, there are five to seven, usually six, spherical swellings.

3. The testis of *D. coeliaca* has been described above. All the members of the genus *Calicotyle* have been described as having numerous testes, except *C. affinis*. Brinkmann (1940) describes *C. affinis* as having a single testicular mass, and he appears, according to

his diagnosis of *Calicotyle*, to regard the structure as a single many lobed testis throughout the genus. Previous authors have regarded each lobe as a single testis. The taxonomic difference between the testes of *Dictyocotyle* and *Calicotyle* cannot be assessed until it is settled whether there are numerous testes or a single testis. If there are numerous testes the presence of vasa efferentia may be expected. None of the authors has mentioned vasa efferentia except Goto (1894), who failed to find any in *C. mitsukurii* and he suggested that irregular cavities in the mesenchyme served as such. The present authors have

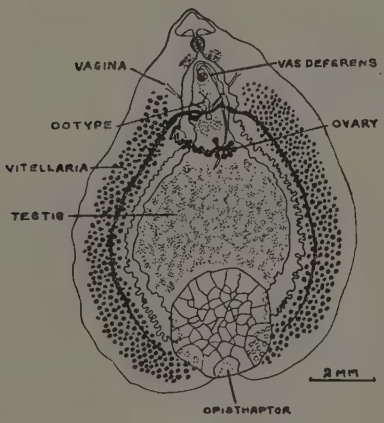

Fig. 7. *Dictyocotyle coeliaca*, adult.

failed to find vasa efferentia in serial sections or whole mounts of *C. krōyeri*.

Sections of *C. krōyeri* also show that the testicular lobes join and their contents may pass from one lobe to another as in *D. coeliaca*. In the whole mount, the testicular mass of *C. krōyeri* appears definitely lobed, whereas in *D. coeliaca* it appears reticulate.

4. The opisthaptor of *D. coeliaca* has been described above. Throughout the genus *Calicotyle* the opisthaptor has seven radial loculi and one central loculus. It projects well beyond the posterior margin of

the body, and has a narrow attachment. A pair of hooks is present on the posterior radial septa in all the species, except *C. inermis*. The musculature of the opisthaptor of *C. kröyeri* is much better developed than that of *D. coeliaca*.

5. The intestinal caeca in *D. coeliaca* end near the posterior margin of the body, well behind the anterior edge of the opisthaptor. The posterior halves of the caeca are sacculated. In *C. kröyeri*, as in other members of *Calicotyle*, behind the middle of the body the intestinal caeca curve inwardly and run horizontally towards the median line for a short distance and then turning sharply posteriorly, end in front of the anterior margin of the opisthaptor, well in front of the posterior margin of the body. There are no sacculations of the caeca in well-extended specimens of *Calicotyle*, except in *C. affinis* where they are extremely shallow.

6. The habitat of *D. coeliaca* is the coelom. All the species of *Calicotyle* inhabit the cloaca or rectum, except *C. inermis* which dwells in the oviducts.

Thus *D. coeliaca* differs from *C. kröyeri*, the type species of the genus *Calicotyle*, in the following characters : (*a*) the position of the opening of the paired vaginae ; (*b*) the termination of the ovary ; (*c*) the structure of the testis ; (*d*) the structure of the opisthaptor ; (*e*) the configuration of the intestinal caeca ; and (*f*) the habitat.

The opisthaptor, the ovary and possibly the testis are the only characters which may be considered to be of more than specific value. It was on the differences of these characters that Nybelin erected a new genus. In referring *D. coeliaca* to the genus *Calicotyle*, Dawes (1948) does not discuss these points.

The genus *Calicotyle* is a very homogeneous group. The ovary and the opisthaptor are remarkably constant in structure, and the latter has been regarded by systematists as an important generic character. It may, of course, be argued that too much importance is attached to the opisthaptor, as variations in the organs of attachment might be correlated with differences in habitat. There can be very little doubt of the close relationship between *Dictyocotyle* and *Calicotyle*. But the differences between the opisthaptor and ovary of *D. coeliaca* and those of *C. kröyeri* appear to be more important than the specific differences within the genus *Calicotyle* (sensu stricto), and at present form a convenient basis for the retention of a distinct separate genus for the former species, thus permitting the homogeneity of the genus *Calicotyle* to remain undisturbed.

The writers consider that the genus *Dictyocotyle* Nybelin, 1941, should for the present be accepted, and the name *Dictyocotyle coeliaca* retained. They wish, however, to state that in accepting the genus *Dictyocotyle* Nybelin, they are not thereby accepting Nybelin's definition of the sub-family Calicotylinae, as this would include the genus *Merizocotyle*, the type of the sub-family Merizocotylinae Johnston and Tiegs, 1922. Furthermore, little advantage is gained from dividing a small family of twelve genera (Monocotylidae) into five sub-families.

Summary.

1. Heavy infestations of the coelom-dwelling trematode, *Dictyocotyle coeliaca*, in rays are reported.

2. Of seven species of skates and rays examined, 16 of 55 specimens of *R. naevus* and 31 of 73 specimens of *R. radiata* were infested.

3. The spindle-shaped, three-sided operculate eggs are described and possible modes of exit from the male and female hosts are discussed.

4. A number of immature stages have been found and descriptions are given. Unlike the adults, the opisthaptor projects prominently beyond the posterior margin of the body, but the number and arrangement of the loculi appears to be the same.

5. The systematic position of the trematode is discussed.

Acknowledgments.

We wish to thank Professor J. Ritchie (in whose department this work was carried out), Dr. D. O. Morgan and Dr. C. Rayski of the Zoology Department, Edinburgh University, for facilities and advice, Professor R. A. R. Gresson, now of Queen's University, Belfast, and Mr. S. Prudhoe of the British Museum, for their helpful criticism; Mr. MacDonald for the photographs and Messrs. Carnie & Gibb, trawler-owners, Granton, for supplying the fresh skates and rays.

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Trichostrongylus leiperi sp. nov., a Parasite of the Eland (*Taurotragus oryx*) in Northern Rhodesia.

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A new member of the genus *Trichostrongylus* Looss, 1905, was collected by the writer from the gastric stomach (abomasum) and the duodenum of an old eland bull in 1931. The animal was shot at a spot amongst the Nega Nega Hills to the east of the Great North Motor Road in the Mazabuka Provincial District.

Eight males and twelve females were collected from the pyloric end of the abomasum which also harboured a large number of *Haemonchus mitchelli* LeRoux, 1929, and a few specimens of *Ostertagia circumcincta* (Stadelmann, 1894). Two more males and four females of the new species were encountered amongst a large number of *Cooperia* spp. including *Cooperia neitzi*, Mönnig, 1931. It was from this animal that the writer recovered the small nematode *Minutrostrongylus taurotragi* LeRoux, 1936, and an *Onchocerca* sp. in intramuscular nodules. The area in which the animal was shot is stocked with cattle and it seems strange that an antelope that will interbreed with cattle should have been parasitized by only one member of the family Trichostrongylidae Leiper, 1912, of cattle and sheep.

For the new trichostrongyle the name *Trichostrongylus leiperi* sp. nov. is designated in honour of Emeritus Professor R. T. Leiper, F.R.S., the founder of the Journal of Helminthology and who, as Director of the Commonwealth Bureau of Agricultural Parasitology, through the Helminthological Abstracts, has kept the helminthologists in the British Mandated Territories, the Protectorates, the Colonies, the Dominions and in other countries informed of developments in a branch of science which is of the utmost importance for the well-being of man, his pets and his domesticated animals in many parts of the world. Professor Leiper has "roamed" and still "roams" the literature for contributions dealing with helminthology as that magnificent, majestic and gigantic antelope, the eland, has roamed the African veld and bush for centuries.

Trichostrongylus leiperi sp. nov.

Although this species is a typical member of the genus *Trichostrongylus* Looss, 1905, the males cannot be mistaken readily for any of the hitherto described members of the genus. The new species appears to be more closely related to *Trichostrongylus vitrinus* Looss, 1905, than to any of the others. Its spicules are relatively slender and like those of *T. vitrinus* are not furnished with very prominent ridges, serrations, hook-like projections or conspicuous spurs and membranous cuticular expansions in the posterior half.

It differs from *T. vitrinus* in that the spicules are more slenderly built and longer. They measure 0·215 to 0·225 mm. and 0·240 to 0·265 mm. in the right and left spicules respectively. Those of *T. vitrinus* are recorded as being 0·160 to 0·170 mm. long. The spicules are slightly sub-equal in length and slightly dissimilar in build as in the species *T. vitrinus*, *T. capricola*, *T. orientalis*, *T. colubriformis*, *T. retortaeformis* and *T. probolurus* which I have examined. Some authorities, including Mönnig (1938) and Clapham (1947) state that the spicules are equal and similar within certain species of some of the above mentioned. I have examined many specimens of the same species but have not yet encountered a single specimen with identical spicules.

In *T. leiperi* the gubernaculum is 0·125 to 0·135 mm. long and 0·085 to 0·095 mm. in length in *T. vitrinus*.

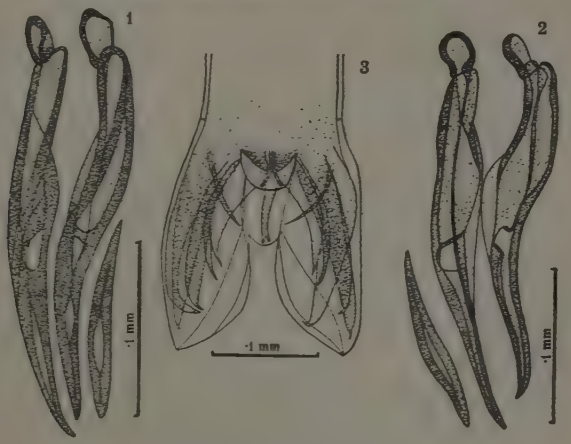
The usual cervical, prebursal and caudal papillae are present. The copulatory bursa is of the usual type and the rays are as illustrated. The genital cone is well developed and its dorsal part is caudally pierced by two ray-like structures (Fig. 3). LeRoux (1936) records the presence of these ray-like structures in *Minutostrongylus taurotragi* and observes that similar structures have been observed in members of the genera *Trichostrongylus*, *Cooperia* and *Ostertagia*. They are present also in other Trichostrongylidae.

The ventral surface of the genital cone bears a minute, rather inconspicuous, appendage which appears to have escaped detection hitherto in *Trichostrongylus* spp. This appendage is homologous to a similarly situated but much better developed appendage in the members of the genus *Cooperia*, and to the V-shaped appendages in the genus *Ostertagia*. These appendages are evidently sensory organs.

The writer considers that his illustrations of the spicules and gubernaculum (Figs. 1 and 2) from two different specimens will suffice to differentiate the new species from *Trichostrongylus vitrinus* Looss, 1905; *Trichostrongylus minor* Mönnig, 1932; *Trichostrongylus thomasi*

Mönnig, 1932 ; *Trichostrongylus capricola* Ransom, 1907 ; *Trichostrongylus orientalis* Jimbo, 1914 ; *Trichostrongylus skrjabini*, *Trichostrongylus colubriformis* (Giles, 1892) ; *Trichostrongylus retortaeformis* (Zeder, 1800) ; *Trichostrongylus axei* (Cobbold, 1879) ; *Trichostrongylus falculatus* Ransom, 1911 ; *Trichostrongylus pietersei* LeRoux, 1932 ; *Trichostrongylus hamatus* Daubney, 1933 ; *Trichostrongylus probolurus* Railliet, 1896 ; *Trichostrongylus rugatus* Mönnig, 1925 ; *Trichostrongylus cervarius* Leiper and Clapham, 1938.

Trichostrongylus longispicularis Gordon, 1933, is omitted from the species described from ruminants because the writer considers it to be a synonym of *Trichostrongylus colubriformis* (Giles, 1892).



Trichostrongylus leiperi sp. nov.

In the Grahamstown area, Cape Province, the writer has collected from Blackheaded Persian sheep specimens of *T. colubriformis* with spicules measuring up to 0·190 mm. in length. Gordon's (1933) illustration of the spicules of *T. longispicularis* appears somewhat diagrammatic. Andrews (1934) reports *T. longispicularis* as a parasite of cattle in the United States.

The writer wishes to observe that he has not succeeded in obtaining a description of *Trichostrongylus mönnigi* Gutteres, 1947, from cattle in Africa.

The chief measurements of the males and females are tabulated in Table I. The type specimens and a few co-types of *Trichostrongylus*

leiperi sp. nov. have been deposited in the helminthological collection at the British Museum (Natural History), London. The remainder of the co-types are in the helminthological collection at this institute.

TABLE I.

The measurements (in micro-millimetres, except where otherwise stated) of the Males and Females of *Trichostrongylus leiperi* sp. nov.

	Males.	*Females.*
Length of Body (in mm.)	4·3–5·1	5·7–7·3
Maximum breadth of body	90–115	93–102
Anterior end to excretory pore	126–165	133–170
Length of oesophagus	600–785	630–825
Diameter of head	10–12	10–14
Length of Spicules—Right	215–240	—
Left	225–260	—
Length of gubernaculum	125–130	—
Vulva to anus	—	1134–1217
Anus to tip of tail	—	66–83
Ovejectors, including sphincters	—	255–366
Eggs in utero—Length	—	72–77
Breadth	—	35–37

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A Trichostrongylid, *Paracooperia mazabukae* sp. nov., from a wild ruminant, the Oribi, in Northern Rhodesia.

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Several specimens of a trichostrongylid, for which the name *Paracooperia mazabukae* sp. nov. is proposed, were collected from the duodenum of the Oribi (*Ourebia ourebi*), a small African antelope, in vicinity of the Nega Nega hills in the Mazabuka area, Northern Rhodesia.

Four species of *Paracooperia* Travassos, 1935, have hitherto been recorded from the small intestine of ruminants. Schwartz (1929) based his description of *Cooperia nodulosa* on specimens collected from nodules in the mucosa of the small intestine of a carabao (*Bubalis bubalis*) in the Philippine Islands. Mönnig (1931) described *Cooperia serrata*, since designated as the type of the genus *Paracooperia* Travassos, from the springbuck (*Antidorcas marsupialis*), not *Damaliscus albifrons* as recorded by Daubney (1933) who records *Cooperia serrata* Mönnig, 1931, as having been collected frequently from sheep in the Rift Valley and the Athi Plains in Kenya. Daubney notes that the normal host of this helminth has not yet been discovered in Kenya. Travassos (1935) treats the Kenya parasite as a new species and proposed the name *Paracooperia daubneyi*. Ortlepp (1939) describes *Paracooperia raphiceri* from the duodenum of a steenbuck *Raphicercus* sp., Pilgrims Rest, Transvaal.

Matoff (1938) identifies as *Schwartziella nodulosa* (Schwartz, 1928) a parasite which he recovered from nodules in the wall of the small intestine and of the caecum of buffaloes in Bulgaria. His illustrations (Figs. 2 and 4) of the spicules of the males from Bulgarian buffaloes show that the caudal extremity of each spicule terminates in a prominent " foot " set at right angles to the long axis of the body of the spicule and not at an angle with the " toe " directed posteriorly as illustrated by Schwartz (1928) for the specimens from intestinal nodules in the carabo in the Philippines.

The characteristic termination of the caudal extremities of the spicules in the specimens recovered by Matoff is such that the species from Bulgaria should be treated as a new species for which the name *Paracooperia matoffi* sp. nov. in now proposed in honour of the discoverer of this helminth.

LeRoux (1936), being unaware of Travassos's (1935) creation of the genus *Paracooperia*, proposed the new genus *Schwartziella* for the species described by Schwartz and by Mönnig.

The examination of the specimens from the oribi revealed that the characters of the genus *Schwartziella*, a synonym of the genus *Paracooperia* Travassos, 1935, as defined by the writer in 1936 should be amended, as far as the characters of the spicules and the presence of a linguiform process in the vulvar region are concerned. The specimens from the oribi, being well preserved, could be orientated readily. This enabled the writer to detect that the dentations or serrations recorded as being present on the medial aspect of the spicules were not true serrations or dentations but were due to the wavy medial chitinous ridge being viewed from a lateral aspect. When this ridge was viewed edge-on its wavy course was detected. It is incorrect to describe the spicules as dentated or serrated. The absence of a prevulvular flap or linguiform process in *P. raphiceri* and *P. mazabukae* necessitates amending the definition of the genus by stating that a prominent linguiform process may be present or absent in the proximity of the vulva.

Paracooperia mazabukae sp. nov.

Characters common to Males and Females. Freshly collected specimens were reddish in colour prior to preservation in boiling glycerine alcohol. Preserved specimens are fairly straight and dark in colour compared with *Trichostrongylus* spp. Cephalic extremity shows a well marked cuticular inflation with prominent transverse striations. In the males the cephalic inflation varied from 0·051 to 0·06 mm. in length by 0·037 to 0·04 mm. in width. The corresponding measurements in the females were 0·05 mm. to 0·075 mm. by 0·036 mm. to 0·044 mm. The rest of the cuticle bears very fine transverse striations and 10–12 longitudinal ridges which increase in number, 14–16, posteriorly. The ridges are low and bear perpendicular striations which give them the appearance of being pectinated. These perpendicular markings correspond with the faint transverse striations on the cuticle. The oral aperture is terminal, small and circular in outline. The head bears six rather inconspicuous cephalic papillae. Inconspicuous cervical papillae are present and are situated at 0·196 mm. to 0·256 mm. and 0·214 mm. to 0·292 mm. behind the anterior extremity in the male and female respectively. The excretory pore is situated at a level of 0·182 mm. to 0·233 mm. and 0·192 mm. to 0·24 mm. from the cephalic extremity in the males and females respectively. The nerve ring was 0·174 to 0·182 mm. and 0·185 mm. to 0·200 mm. caudal to the head in

the male and female respectively. The oesophagus is of the type met with in the Trichostrongylidae. In the male it is 0·288 mm. to 0·324 mm. long and in the female 0·306 to 0·366 mm.

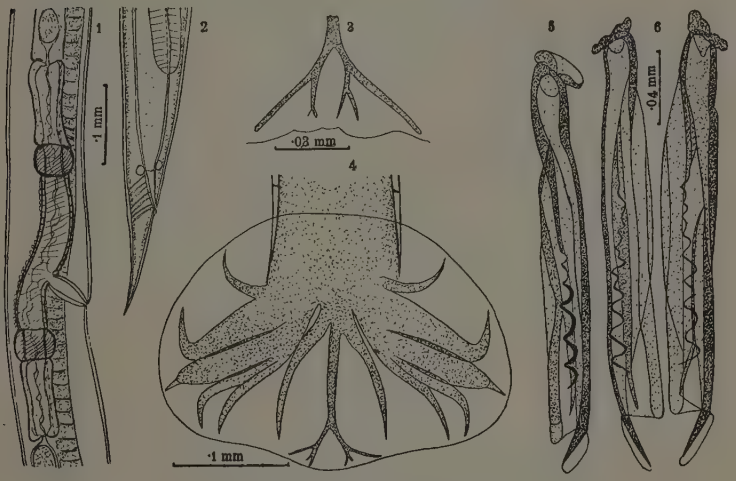
Female Characters. The specimens which were measured varied in length from 6 mm. to 6·9 mm. with a maximum breadth of 0·085 mm. to 0·103 mm.

The tail (Fig. 2) attenuates uniformly and terminates in a fine point at 0·122 mm. from the anus. It bears the usual caudal papillae. The vulva, situated at 1·15 mm. to 1·44 mm. from the caudal extremity, is a transverse slit with moderately protuberant lips and without a linguiform process as recorded for *P. nodulosa*. *P. serrata* and *P. daubneyi*. The vagina is short and is directed antero-medially. Ovejectors and uteri (Fig. 1) are divergent as figured. The eggs, *in utero*, are oval, thin-shelled and measure 0·050 mm. to 0·056 mm. by 0·034 mm. to 0·038 mm.

Male Characters. The length of the males varied from 4·5 mm. to 5·8 mm. and the maximum breadth was 0·56 to 0·78 mm. The copulatory bursa is voluminous and is not divided into distinct dorsal and lateral lobes. Ventral surface of the bursa is ornamented with small bosses. The bursal rays are well developed and disposed as illustrated (Figs. 3 and 4). The spicules are not absolutely identical in conformation and length. They are relatively massive in appearance, brownish in colour, well chitinised and furnished with ridges and cuticular expansions as illustrated (Figs. 5 and 6). Attention has already been drawn to the fact that they are not serrated or dentated. The most caudal and main termination or " foot " of the spicule is directed caudally and slightly medially and bears a cuticular expansion. The exact disposition of the cuticular expansion could not be determined with certainty in spicules lodged within the body of the worm. In this genus as in the genera *Haemonchus*, *Trichostrongylus*, *Cooperia* and others the exact disposition of the ridges and cuticular expansions can probably be best ascertained by allowing the newly collected males to remain in distilled or tap water until the spicules are expelled from the body of the worm. They may also be removed by careful dissection with very finely pointed needles. The " foot " in the new species seems much longer and more attentuated at the " toe " than in the hitherto described species. A gubernaculum was not detected but the genital cone carries a structure the exact extent and nature of which could not be determined.

Affinities. The species from the oribi is most closely related to *Paracooperia raphiceri* Ortlepp, 1939, which has no linguiform process

in the region of the vulva. The fact that the writer has observed a poorly developed linguiform process in specimens of *Trichostrongylus colubriformis* and that this structure may be absent or present in varying degrees of development in members of the genera *Haemonchus*, *Ostertagia* and others, suggests that little specific significance can be attached to them in the differentiation of species.



Paracooperia mazabukae, n.sp.

Fig. 1. Vulvar region of female. Fig. 2. Tail of female. Fig. 3. Dorsal ray of bursa. Fig. 4. Ventral view of bursa. Fig. 5. Spicule showing so-called serrations. Fig. 6. En face view of ridges in left and right spicules.

It seems to be generally accepted that the length of the spicules and the characters of the ridges and cuticular alae on them are valid features on which the differentiation of species within a genus can be based.

The species from the oribi can be distinguished from *P. raphiceri* by the number and the extent of the so-called " serrations " on the spicule. In the new species, described here, the ratio of the length of the " serrated " or " dentated " part of the spicule to the total length of the spicule, caudal cuticular inflation not included, varies between 1 to 2·5 and 1 to 3. In the other above-mentioned species this ratio,

estimation based on the drawings of the spicules, are :—

in *P. daubneyi* and *P. raphiceri*	... 1 to 4
P. serrata	... 1 to 6
P. nodulosa	... 1 to 2·7 and 1 to 3·3
P. matoffi	... 1 to 2·8

The number of " serrations " or " dentations " are stated to number 4 in *P. raphiceri*, 4 to 5 in *P. serrata* (5–6 according to LeRoux 1936), 8 to 12 in *P. matoffi*, and 7 in *P. daubneyi* and *P. nodulosa*. The drawings of the spicules in the last-mentioned species suggest that the prominent "dentations" may number 7 but that less well-developed dentations are present and that there appear to be 9 on one spicule and 11 on the other. In *P. mazabukae* the number of " serrations " varied from 6–10 and in only one worm were the same number, 7, observed on both spicules. In the other specimens the number were : 7 & 6, 8 & 6, 8 & 7, 8 & 7, 9 & 7, 9 & 8, 9 & 8, 10 & 8, 10 & 9, 11 & 9.

The degree of development of the " serrations " or " dentations " varied considerably on the same spicule. The most prominently developed were caudally situated. It has already been stated that the recorded serrations or dentations are merely caused by viewing a wavy chitinous ridge in one optical plane from a lateral aspect.

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On the Systematics of *Syngamus trachea* (Montagu, 1811) Chapin, 1925.

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The gapeworm is of great economic interest, as it often menaces the successful rearing of poultry and game birds. Since the parasite was found in the trachea of several of these birds examined by me, the opportunity was taken to carry out researches into the systematics of the genus *Syngamus*. Such researches seemed necessary because of considerable disagreement in the literature concerning the delimitation of several species recorded from birds. In addition to my own material, I have also examined that belonging to certain helminthological collections in Copenhagen (see p. 40). Finally, the conclusions of earlier investigators have been substantiated, and new ones have been found to be important in judging the epidemiology of the species.

This parasite, which is easily recognizable in the trachea of birds, has been recorded from a large number of avian host-species. It was originally found in poultry by Wiesenthal (1799), who merely referred to it as a "worm of poultry." Later, as *Fasciola trachea* it was recorded from pheasant and partridge by Montagu (1811). But it was left to von Siebold (1836) to recognize the worm as a nematode (*Syngamus trachealis*) and to provide the first description of its anatomy. This description was probably based on the specimens from poultry, although he also recorded the parasite from *Picus viridis* and *Apus apus*. In the light of modern knowledge the worms from *Picus* and *Apus* require further investigation, although their occurrence in these hosts is not improbable, since *S. trachea* has been recorded from birds belonging to several families. Nathusius (1837) recorded "*Strongylus trachealis*" in *Ciconia nigra*, but von Siebold (1837) demonstrated that this worm belonged to another species, now known as *Cyathostoma variegatum* (Creplin, 1849). Nevertheless, *Ciconia nigra* and, probably by mistake, *Ciconia ciconia* have been mentioned in lists of the hosts of *Syngamus trachea*, even up to the present time. Dujardin (1845) accepted the genus *Syngamus*, and since his time the species had generally been called *Syngamus trachealis* until Chapin (1925) revised its nomenclature.

In some cases the name without any apparent reason was changed, e.g. Creplin (1849), Schlotthauber (1860), Molin (1860), Theobald (1896), (see list of synonyms below) ; and Diesing (1851), who did not accept

the genus *Syngamus*, called the species *Sclerostoma syngamus*, a name which has also been used by some other authors. Chapin (1925), following the rules of nomenclature was the first to establish the now current name of *Syngamus trachea* (Montagu, 1811).

In addition, Chapin (1925) described two new species: *S. parvus* and *S. gracilis*, from *Nucifraga caryocatactes* and *Corvus brachyrhynchos* respectively. Manter and Pinto (1928), in describing *Syngamus tenuispiculum* from *Turdus migratorius* suggested in a foot-note that their species might be identical with *Syngamus trachea*. This suggestion was adopted by Manter's pupil Ripple (1941), but Goble and Kutz (1945) doubted this determination and considered the species to be identical with *S. merulae* Baylis, 1926.

The main characters on which these species had been separated were divergent measurements of various organs of the body, differences in the lengths of the spicules and variations in the branching of the bursal rays. Lewis (1928), in a very important paper, showed the astonishing variability of just those characters, which have been considered systematically important.

The dorsal ray in *Syngamus trachea* is generally described as double tridigitate, whereas in other species it is described as double or merely bifurcated. As is especially demonstrated by Lewis (1928), and fully corroborated by myself, this may vary even in worms from the same host specimen (see Fig. 1, a–h).*

The spicules of worms from different hosts are similar in shape, although in general appearance they may vary very considerably, but in no way constantly (see Fig. 2, a–e, h–m). The spicules are short, equal or subequal in length, sometimes the right, sometimes the left spicule being the longer. I have found that the greatest discrepancies between the length of the spicules in a single specimen corresponds with an index between the longer and shorter as large as 1 : 2·1. The measurements of the lengths of 29 pairs of spicules varied between 60μ and 101μ, the most common figure being about 80μ. The lengths most commonly given for *S. trachea* vary between about 60μ and 70μ, but Cobbold (1861) found spicules measuring 113μ, and a length as low as 49μ has been recorded. Chapin (1925) is of opinion that the reports of spicules measuring 140 μ probably refer to another species. These latter reports I have been unable to trace, but I think it quite

* Altogether the variability in the bursal rays in Strongyloid nematodes does not seem always to have been duly recognized in the current descriptions (compare f.i. Pereira and Vaz (1930), Dikmans (1936)).

probably that such sizes actually do exist in particularly large specimens.

Chapin (1925) thought that Syngamids in Corvid birds, previously referred to *S. trachea*, would prove to be his *S. gracilis*. This has later been demonstrated not to be the case, because numerous cross-infection experiments with material, undoubtedly belonging to *S. trachea*, from domestic and game birds, and with *Syngamus* from Corvid birds and

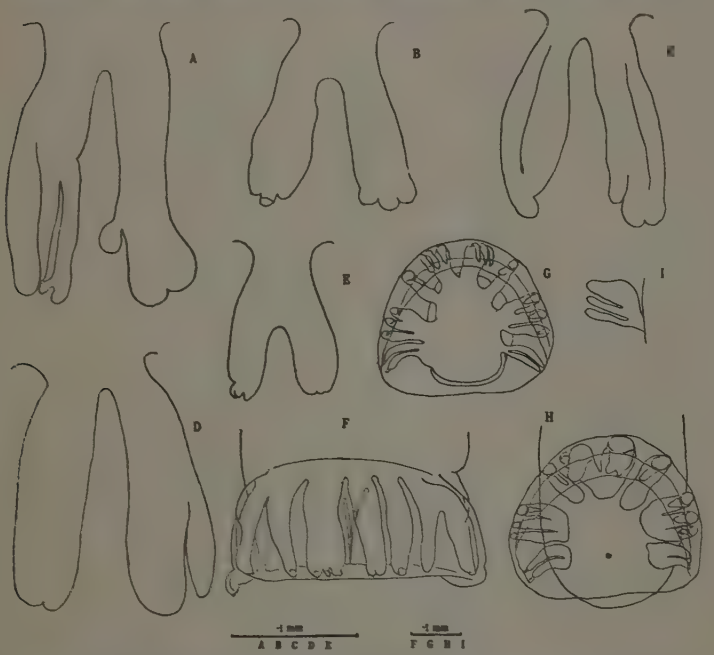

Fig. 1. Bursal rays in *Syngamus trachea*.

A and D: Dorsal ray of specimens from *Corvus frugilegus*. B and C: Dorsal ray of specimens from *Gallus domesticus*. E: Dorsal ray of specimen from *Turdus merula*. F: Bursa and spicules, dorsal view, of specimen from *Garrulus glandarius*. G and H: Bursa, ventral view, of specimens from *Gallus domesticus* and *Melancorypha calandra*, respectively. I. Lateral ray of right side, dorsal view, of another specimen from *M. calandra*.

Starlings, conclusively prove that *Syngamus trachea* is capable of infesting a large number of species of birds, e.g. Clapham (1934, 1935, 1938, 1940), Leiper (1926), Lerche (1928), Morgan and Clapham (1934), Rice (1929), Szidat (1928), and Taylor (1928, 1935, 1938); Goble and Kutz (1945) have also arrived at a similar conclusion.

The last-mentioned authors also doubt the validity of *S. parvus*. The only characters distinguishing this species are the short spicules and the peculiar shape of the lateral ray of the bursa. Considering the possibility of considerable variation in spicule-length this difference cannot be decisive, and the shape of the lateral ray is rather similar to a ray found by me in a specimen from *Melanocorypha calandra* (Fig. 1, i). I think it is justifiable, therefore, to regard *S. parvus* as a synonym.

Syngamus merulae originally described from *Turdus merula*, might seem to be a valid species. I have examined specimens of this parasite, which is easily recognized by the absence of an annular thickening at the anterior end of the mouth capsule, a feature found by a number of authors, e.g. Baylis (1926), Manter and Pinto (1928), Clapham (1934), Goble and Kutz (1945) (see Fig. 3, c, e–f). On the other hand, the shape of the mouth capsule is apparently very variable. During my researches into *Syngamus* from Gallinaceous and Corvid birds, I was inclined to think at one time that the worms from the latter might represent a distinct species, owing to certain differences in the shape of the mouth-capsule and the broad cuticular rim round the mouth (Fig. 3, a–b, d), but a closer comparative examination showed that they all belonged to the same species. Not infrequently the mouth capsule, especially in the male, has a tendency to be reduced, and then the shape becomes very similar to that of the *Syngamus* from species of *Turdus*. But the most convincing argument against the distinctiveness of *S. merulae* is, in my opinion, that Walker (1897) has been able to infest robins (*Turdus migratorius*) with *Syngamus* from chicks, and vice versa. Similar results have been obtained from experiments carried out by Ripple (1941). Clapham (1934) also succeeded in infesting chicks with *S. merulae*. Both Walker and Ripple mention the presence of the cuticular rim in their specimens. On the other hand, Goble and Kutz (1945) have seen a single pair of worms from Ripple's material, in which this rim is absent. These facts make it probable that this rim is usually not developed in the form in Turdid birds. I therefore consider *S. merulae* also to be a synonym of *S. trachea*.

In a few cases only one spicule appeared to be present, evidently owing to fusion of the usual pair (Fig. 2, f–g). That fusion actually does occur, is clearly demonstrated by partial fusion observed on one occasion (Fig. 2, e). On the whole there is in this genus a strong tendency to fusion. Von Siebold, in his first paper was of the opinion that the male and female were coalescent, really being a " Doppeltier,"

like *Diplozoon paradoxum*. Subsequently (1837), he stated that they are only strongly connected, and observed that the eggs escape from below the bursa of the male. In later literature, however, it was often stated that the eggs could only be freed through bursting of the mother's body, an assumption probably based solely upon von Siebold's original

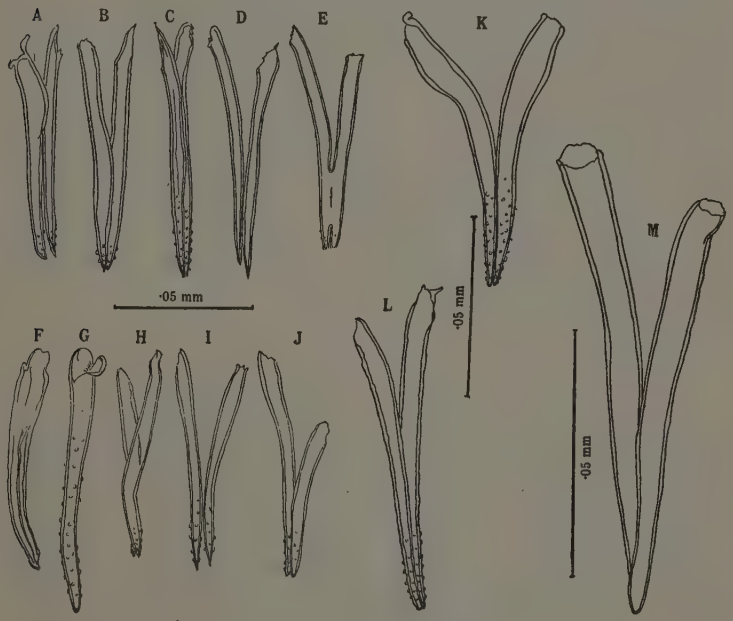

Fig. 2. Spicules in *Syngamus trachea*.

A: Specimen from *Perdix perdix*, left dorso-lateral view. **B**: From *Phasianus colchicus*, ventral view. **C**: From *Anser anser dom.*, lateroventral view. **D**: From *Turdus merula*, dorsal view. **E**: From *Lyrurus tetrix*, left latero-dorsal view, partly fused. **F**: From *Turdus merula*, fused. **G**: From *Anser anser dom.*, fused. **H**: From *Larus canus*, viewed from left. **I**: From *Corvus frugilegus*, dorsal view. **J**: From *Pica pica*, dorsal view. **K**: From *C. frugilegus*, dorsal view. **L**: From *C. frugilegus*, viewed from right. **M**: From *Garrulus glandarius*, ventral view.

statement. Several subsequent authors have observed the eggs escaping from beneath the bursal border, and this occurrence is substantiated by the common finding of eggs in faeces. Szidat (1928), on the other hand, was of opinion that the worms occasionally were

actually fused, and during the present investigation instances occurred in which it was quite impossible to separate the sexes without tearing them to pieces—a condition often noted previously. In such instances only histological examination can decide the question. Fusion is not a constant phenomenon, however, because in all species, including those from mammals, a bursa has been described. Possibly the fusion may be partial, and therefore allows the eggs to pass. A similar tendency towards fusion presents itself in the bursal rays, and in the variable number of teeth in the buccal capsule; or, as in several mammalian species, in the presence of the dentigeral ridges. All this occurs simultaneously with the above-mentioned tendency of reduction, which is also apparent in the reduction of the spicules in comparison with the genus *Cyathostoma*, in which the connection between the sexes is not as close as in *Syngamus*. In some mammalian species the spicules are rather faintly developed, and in some instances may or may not be present; cf. Chapin (1924), Buckley (1934), Vaz (1935), Lent and Penna (1939).

On the basis of the above considerations the following synonymy can be given.

Syngamus trachea (Montagu, 1811) Chapin, 1925.

Synonymy and main references: " Worm of poultry," Wiesenthal, 1799, p. 204; *Fasciola trachea* Montagu, 1811, p. 149, Pl. 7, Fig. 4; *Distoma lineare* Rudolphi, 1819, p. 113–114, 414–415; *Syngamus trachealis* von Siebold, 1836, p. 106, Pl. 3, Fig. 1–2; *Strongylus trachealis* Creplin, 1846, p. 131; (*Non Strongylus trachealis* Nathusius, 1837= *Cyathostoma variegatum* (Creplin, 1849)); *Syngamus trachealis* Dujardin, 1845, p. 261–262, p.p.; *Strongylus pictus* Creplin, 1849, p. 64; *Sclerostomum syngamus* Diesing, 1851, p. 302–303; *Sclerostomum tracheale* Diesing, 1851, p. 303, p.p.; *Syngamus primitivus* Molin, 1860, p. 565–566; *S. pugionatus* Schlotthauber, 1860, p. 127 (nomen nudum); *Sclerostomum syngamus* Cobbold, 1861, p. 305, Fig. 1–6; *Syngamus trachealis* Mégnin, 1881, p. 45–67, 2 Pl.; *S. bifurcatus* Theobald, 1896, p. 74; *S. trachealis* Walker, 1897, p. 64–65, Pl. 1, Fig. 1–8; Hutyra and Marek, 1913, II, p. 89; Skrjabin, 1916, p. 464–467; Ortlepp, 1923, p. 119–140, Fig. 1 B; *S. trachea* Chapin, 1925, p. 563–564, Pl. 1, Fig. 2, Pl. 2, Figs. 8–10, 18, 20; *S. parvus* Chapin, 1925, p. 560–562, Pl. 1, Fig. 1, Pl. 2, Fig. 15, 19. *S. gracilis* Chapin, 1925, p. 562–563, Pl. 4, Figs. 38, 40, 42; *S. merulae* Baylis, 1926, p. 661–665, 4 Figs.; *S. trachea* Yorke and Maplestone, 1926, p. 157, Fig. 101, a–c; Cram, 1927, p. 34–38, Fig. 36–39; *S. gracilis* Cram, 1927, p. 39–41, Fig. 44–46; *S.*

parvus Cram, 1927, p. 39, Fig. 41–43 ; *S. trachea* Lewis, 1928, p. 99–112, 2 Figs. ; *S. tenuispiculum* Manter and Pinto, 1928, p. 454–456, Pl. 60, Fig. 1–12 ; *S. trachea* Sprehn, 1932, p. 498–499 ; *S. parvus* Sprehn, 1932, p. 700 ; *S. trachea* Baylis, 1936, p. 312–314, Fig. 144–145 ; Neveu-Lemaire, 1936, p. 968–973, Fig. 496–497 ; Wehr, 1937, p. 77, Fig. 1, 14 ; Holger Madsen, 1941, p. 30–31 ; Ripple, 1941, p. 370–371 ; Goble and Kutz, 1945, p. 394–399 ; *S. parvus* Goble and Kutz, 1945, p. 399 ; *S. merulae* Goble and Kutz, 1945, p. 399.

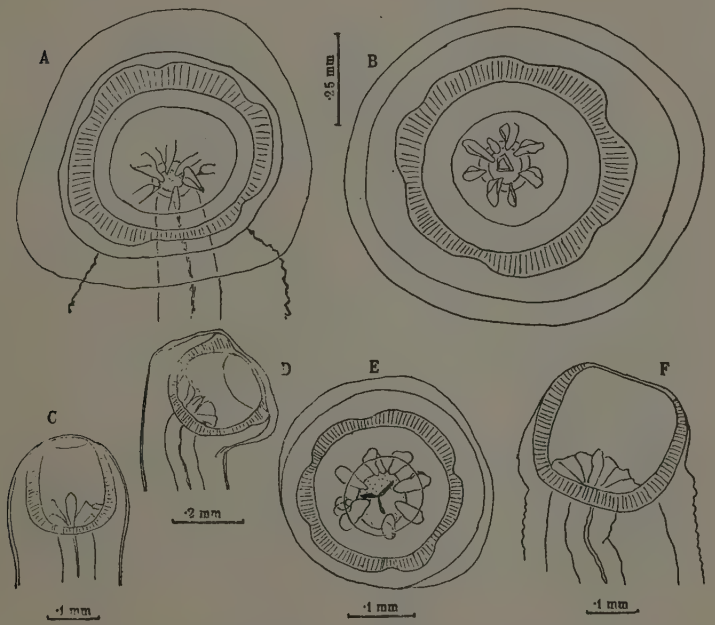

Fig. 3. Mouth capsules of *Syngamus trachea*.
A and B : Specimens, female, from *Corvus frugilegus*. C : Specimen, male, from *Turdus merula*. D : Specimen, male, from *C. frugilegus*. E and F : Specimens, female, from *T. merula*.

Besides *S. trachea* and the synonyms mentioned above, the following names of species of *Syngamus* from birds can be found in the literature : *S. coelebs* Schlotthauber, 1860, occurring in *Buteo lagopus*, and *S. mucronatus* Schlotthauber, 1860, in *Picus canus* and *Dryobates major*.

Both these species are nomina nuda. Possibly the *Syngamus* from the above hosts will prove to be *S. trachea.*

Further, there is *S. microspiculum* Skrjabin, 1915, from *Phalacrocorax carbo,* this host not being a Corvid bird, as presumed by Clapham (1940) and Goble and Kutz (1945). *S. microspiculum* appears to be a distinct species. The number of its buccal teeth is given as three, as against 8 (6–10) commonly found in *S. trachea.* Judging from Skrjabin's drawings, buccal ribs also occur, as in the mammalian species, *S. nasicola* v. Linstow, 1899 and *S. laryngeus* Railliet, 1899 (see Buckley, 1934). Moreover the shape of the spicules, and of the eggs, differs from that of *S. trachea.* The length of the spicules in *S. microspiculum* has been much discussed, owing to inaccuracies in the descriptions given by Skrjabin (1915 and 1916). In his 1915 paper he gives the length as 150μ, but in 1916 as 115μ. On the ground that in his description of *S. trachea* Skrjabin gives the length of the spicules as 690μ instead of 69μ, Manter and Pinto (1928) suggest that the length in *S. microspiculum* may be 11.5μ. Clapham (1940), by some obscure calculation, arrived at a length of 49μ. Measurements taken from Skrjabin's drawing give a length of 108μ (not 88–90μ, as Goble and Kutz indicate), rather confirming his statement of 1916. The species at any rate requires further investigation. I have myself examined 270 cormorants in order to find the species, but without success. Possibly the *S. trachea* mentioned by Skrjabin (1915) from *Pelecanus onocrotalus* (source ?) and noted in several lists of hosts, belongs to *S. microspiculum.**

Finally, a species of *Syngamus* from the owl, *Carine noctua,* has been described by El'perin (1938), whose paper is not available to me.

In Denmark, *S. trachea* has previously been recorded from fowls, Hansen (1904–05), Lænkholm (1924–25) and from pheasants, Hansen (1904–05), Christiansen (1935–36). In my own investigations I have found it in pheasant, partridge and black grouse ; rook, hooded crow, jackdaw, magpie and blackbird. On one occasion I found young specimens without eggs in a *Larus canus* (see Figs. 2, h). In the collections of the Royal Veterinary Agricultural College, Copenhagen, there are specimens from other hosts, namely : starling, turkey, jay, and *Melanocorypha calandra,* the latter from the Copenhagen Zoological Gardens. The Zoological Museum of the University of Copenhagen possesses specimens from fowls. Finally, I received from Dr. Hans

* The explanation is possibly to be found on p. 163 in v. Linstow's Compendium (1878), where *Pelecanus onocrotalus* is mentioned as host for *Sclerostomum pelecani* Chatin = *Gnathostoma pelecani* (Chatin, 1874), according to Cram (1927, p. 365). The name *Sclerostomum* is most commonly used for Syngamidae and near relatives.

Roth of the Laboratory of Veterinary Pathology, Copenhagen, specimens from a gosling. In Norway *S. trachea* has been recorded from fowls and turkeys, Horne (1910), and from capercailzie, Lund (1946). In Sweden it has been found by Hülphers, Lilleengen and Henricson (1941, 1943, 1944), in black grouse, partridge, pheasant, capercailzie and "grouse." In the collection of the Naturhistoriska Museet, Göteborg, there are specimens from a jay.

On the basis of the literature, my own records and conclusions regarding synonymy, already cited, the following revised host-list for **Syngamus trachea* can be given (new hosts being marked with an asterisk).

Passeriformes: *Corvus corone cornix, C. c. corone, C. frugilegus, Coloeus monedula, Pica pica. Nucifraga caryocatactes* (Chapin, 1925: *S. parvus*). *Garrulus glandarius. Pyrrhocorax graculus* (v. Linstow, 1889, p. 89) (?). *Corvus brachyrhynchos* (Chapin, 1925, Canavan, 1931: *S. gracilis* Goble and Kutz, 1945). *Heteropsar albicapillus* Blyth (Johnston and Mawson, 1941: *S. gracilis*). *Sturnus vulgaris. Passer domesticus* (Campbell, 1935; also experimentally: Railliet, 1901, Cuvillier, 1932), *Carduelis cannabina, Delichon urbica* (see Lewis, 1925). *Parus major, Serinus canarius* (experimental infestations; the latter species also Railliet, 1901), *Richmondena cardinalis, "Euplectes melanogaster"* (= *Euplectes taha* ?) (confined birds), (Ehlers, 1872). *Padda oryzovora* (experimental infestation) (Railliet, 1901). *Cyanocitta cristata* (confined) (Klee, 1895, without source). *Zonotrichia leucophrys gambeli, Seiurus noveboracensis, Junco hyemalis* (Cram, 1930). *Sturnella magna, Quiscalus versicolor* (Goble and Kutz, 1945), *Turdus merula* (Baylis, 1926: *S. merulae*), *Turdus ericetorum* (Lewis, 1925, Campbell, 1935: *S. merulae*), *Turdus musicus* (Campbell, 1935: *S. merulae*). *Turdus migratorius* (Walker, 1886, Manter and Pinto, 1928, Ripple, 1941. Goble and Kutz, 1945: *S. merulae*). **Melanocorypha calandra* (confined).

Piciformes: *Picus viridis* (v. Siebold, 1836, Stölker, 1872) (?). *Picus canus, Dryobates major* (Schlotthauber, 1860: *S. mucronatus*, nomen nudum) (??).

Apodiformes: *Apus apus* (v. Siebold, 1836) (?).

Strigiformes: *Athene noctua* (confined), Stölker, 1872) (?).

Psittaciformes: *Nymphicus hollandicus* (experimental infestation) (Mégnin, 1882).

Columbiformes: *Columba livia dom.* (Rossi, 1906).

* In the future this parasite will no doubt be found in a further variety of birds, especially in zoological gardens. Some additional hosts may be found in the paper of Grosso, Prieto & Strobino (1944), which I have been unable to consult.

Charadriiformes : **Larus canus*. *Calidris maritima* (Campbell, 1935). *Vanellus vanellus* (Cobbold, 1861). *Rostratula bengalensis* (Srivastava, 1937).

Gruiformes : *Otis tarda* (Grosse, 1908).

Galliformes : *Tetrao urogallus* (Stölker, 1872, (confined bird), Galli-Valerio, 1935, Clapham, 1940, Hülphers, etc., 1941–44, Lund, 1946). *Lyrurus tetrix* (Galli-Valerio, 1935, Hülphers, etc., 1941–44, own find). *Lagopus lagopus scoticus* (Shipley, 1909, Clapham, 1940, Wehr, 1940). *Bonasa umbellus, Lophortyx californica* (Wehr, 1940, Herman, 1945). *Colinus virginianus* (Webster and Addis, 1945). *Alectoris rufa* (Mégnin, 1882, Clapham, 1940), *Alectoris graeca* (Clapham, 1940, Galli-Valerio, 1939, Herman, 1945). *Perdix perdix. Coturnix coturnix* (Mégnin, 1896, from Rosso, 1906). *Gallus domesticus. Gallus gallus bankiva* (Kraneveld and Douwes, 1940). *Phasianus colchicus. Syrmaticus reevesi* (Smith, 1908). *Chrysolophus pictus* (Thierry, 1869, from Klee, 1899 and Skrjabin, 1916). *Pavo cristata, Numida meleagris, Meleagris gallopavo dom., Meleagris gallopavo* (Wehr, 1940).

Falconiformes : *Falco tinnunculus* (Baylis, 1939). *Buteo lagopus* Schlotthauber, 1860 : *S. coelebs* (nomen nudum ??).

Anseriformes : *Anser anser dom.* (several veterinary handbooks, Wehr, 1940, own record), *Anas platyrhynchos dom.* (Hayem, 1875, from Rossi, 1906 ; several veterinary handbooks. See e.g., Ebcr and Pallaske-Eber (1934)).

Summary.

1. *Syngamus parvus* Chapin, 1925, *S. gracilis* Chapin, 1925, and *S. merulae* Baylis, 1926, are shown to be synonyms of *S. trachea* (Montagu, 1811). These conclusions are important in judging the epidemiology of the species.

2. The list of hosts has been critically revised and supplemented with two new hosts, *Melanocorypha calandra* and *Larus canus*.

Addendum.

In a recent paper by Ta Hsiung Chin (1950) *Syngamus hexadontus* is described as new. He apparently examined only two specimens. Nothing in the description given seems to differentiate it with certainty from *Syngamus trachea*. The chief differential characters of the new species are as follows. 1. Six teeth in the buccal capsule. (The same number often occurs in *S. trachea*). 2. The shape of the dorsal bursal ray. (Considering the enormous variability of this feature in *S. trachea*, the shape described in *S. hexadontus* cannot be regarded as differing significantly from those which have been illustrated in *S. trachea*). 3. The spicules. (These resemble very closely, both in shape and size, those of *S. trachea*). As a further characteristic he finds the tail end of the female more sharply pointed than in *S. trachea*. This, however, does not seem to be the case when comparison is made with the illustrations given by Lewis (1928, fig. 1, p. 103). The only

feature which suggests that there might be a difference is the egg; but this is figured in too small a magnification to be assessed with certainty. Moreover, the dimensions of the eggs lie within the range found in *S. trachea*. There are no dentigeral ridges in the buccal capsule, as seems to be the case in the other species of *Syngamus* found in a cormorant, namely, *S. microspiculum*, from which it also differs in the shape of the eggs.

The taxonomical status of *Syngamus hexadontus* is, therefore, uncertain. Considering the bionomics of cormorants it is curious that they should become infested with *S. trachea*, though it is certain that the worm would be able to thrive in this host. If *S. hexadontus* really proves to be identical with *S. trachea*, its occurrence in cormorants suggests that the infestation must have been acquired during the collecting of nest material.

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A Technique for Counting Trematode Eggs in Sheep Faeces.

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The eggs of the common trematode parasites of ruminants in Australia, *Fasciola hepatica*, *Paramphistomum* spp. and *Cotylophoron cotylophorum* do not float readily in the sugar, brine or glycerine solutions which are commonly used to float other helminth eggs.

A dilution-count technique, for example that devised by Stoll (1930), is satisfactory if many eggs are present and if time can be spared to traverse the whole area covered by a $\frac{7}{8}$ sq. in. cover-slip. However, commonly less than 1,000 and very often less than 200 trematode eggs are present in a gramme of faeces, so that a concentration technique is essential.

Several techniques for the detection and enumeration of trematode eggs in faeces have been described but most of them require considerable time and depend upon concentration by sedimentation (Rivas, 1928 ; Alicata, 1941 ; Olsen, 1946 ; Van Someren, 1947).

Special methods for the diagnosis of schistosomiasis have been devised or described by a number of workers, including Barody (1948), and Hunter (1948).

Methods for concentrating trematode eggs by floatation have been described by several authors. For example, Thienel (1925), Vajda (1927), and Nöller and Schmid (1927), used waterglass ; Brugemann (1937) compared waterglass with concentrated KOH, with or without added sugar ; Lehrke (1941) compared potassium mercuri-iodide with waterglass and with potassium carbonate and Kotlan and Vajda (1941) used potassium carbonate.

Apparatus and Materials.

1. *The Mixing Pipette.*—The floatation fluid used in these experiments (a solution of K_2HgI_4) is expensive and the smallest quantity which is satisfactory for the purpose is used. To achieve this, a special pipette (Fig. 1) was designed for sampling and mixing two equal volumes of fluids of different specific gravity.

The pipette, designed to take up and mix approximately 1.8 ml., was made from soda-glass tubing of 0.5 cm. bore. It has a constriction (A) 3 cm. from the delivery end (E) and, above this, a mixing bulb (B)

approximately 2.5 cm. in diameter and set off-centre. There is a second constriction (C) above the mixing bulb.

The dimensions are proportionately increased and tubing of larger bore is used if delivery of 2 ml. is required (see 2 (*b*)). It should be noted that the capacity of the pipette is slightly in excess of the volume to be delivered. This is convenient, and does not matter because measurement of the volume of suspension in which the eggs are counted is made in the chambers of the counting slide or sliding-top apparatus.

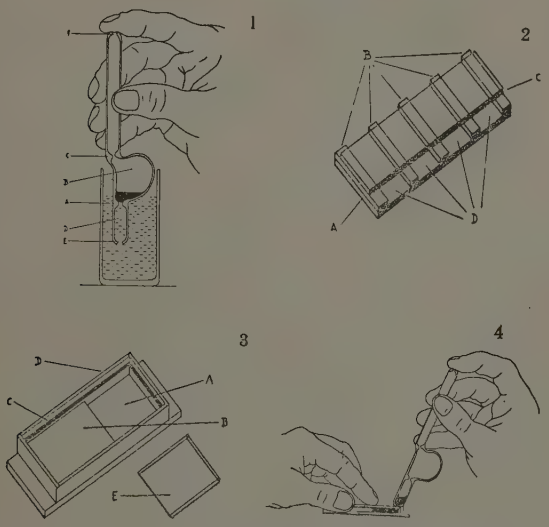
The pipette is made from a length of soda-glass tubing of 0.5 cm. bore which is drawn to a spindle and sealed. A pointed flame is used to form two constrictions, 4 cm. apart, having a lumen of 1–1.5 mm. The tubing between the constrictions is blown to form three small bulbs which are then fused together into one large bulb. While molten, the bulb is allowed to drop off centre, and is then blown to approximately 2.5 cm. diameter. Care is taken to keep the tubing on either side of the bulb parallel, but not in direct alignment, one being slightly above the other (Fig. 1). The tube is cut to leave a stem 3 cm. long at one end of the measuring bulb (D), and a stem 7 cm. long at the other end. The cut ends are rounded in the flame to provide openings 3 mm. in diameter, (E) and (F).

2. *The Counting Chambers.* (*a*) *The counting slide* (Fig. 2).—This resembles the original McMaster egg-count slide (Gordon and Whitlock, 1939), but has different proportions. The base (A) is a glass slide 7.5 cm. × 3.0 cm., upon which are mounted, by means of the glass cement described by Whitlock (1948) five cross pieces (B), each 5 mm. wide, 3.0 cm. long, and 2.0 mm. thick. All surfaces of the cross pieces must be at right angles and should be polished. The cross pieces are set 12.5 mm. apart. The top piece (C) is 7.5 cm. × 2.2 cm., and bears on its under surface parallel engraved lines, 20 mm. apart. The top piece is cemented to the cross pieces off-centre, to leave a filling platform (D). With these dimensions, each chamber contains between the engraved lines a volume of 0.5 ml. (12.5 × 20 × 2 mm.).

(*b*) *The sliding-top apparatus* (Fig. 3).—The apparatus described by Whitlock (1941) for counting small numbers of nematode eggs in sheep faeces will accommodate 2 ml. if the flotation chamber (A) is made 20 × 25 × 4 mm. deep. The platform B is set 0.3 mm. below the sliding ledges (C), to eliminate the support strips on the sliding top in the original apparatus. The apparatus should be made entirely of glass to prevent inaccuracy due to buckling of celluloid. Celluloid was used for the original apparatus (Whitlock, 1941). The apparatus is cemented together without the outer walls (D). The trough (A) is then

filled from a calibrated pipette to determine its capacity which at this stage is usually slightly in excess of 2 ml. The capacity can be reduced to 2 ml. by grinding the upper surfaces of the sliding ledges (C) before the outer walls (D) are cemented to their sides.

3. *The Floatation Fluid.*—A potassium mercuri-iodide (K_2HgI_4) solution is prepared by dissolving 32 gm. KI in 90 ml. distilled water and adding 50 gm. HgI_2, heating gently if necessary. The specific gravity of the solution is approximately 1.63.



Figs. 1 to 4. Egg-counting apparatus and procedure.

METHODS.

Because trematode eggs sink very rapidly in watery suspensions and rise rapidly in potassium mercuri-iodide solution, it is imperative that all manipulations be carried out as quickly as possible.

1. *The Counting Slide.*—A sample of 2 or 4 gm. of faeces is allowed to soak in 50 ml. tap water until thoroughly broken down. An even suspension is prepared by mixing, either with the electric stirring

device described by Kauzal and Gordon (1941) or by shaking with glass beads in a strong jar of convenient size.

Immediately after mixing, the pipette, held vertically and with the bulb towards the hand, is lowered into the faecal suspension until the suspension rises to the constriction in the tube (A). With a finger over the upper end, the pipette is then lifted from the faecal suspension. The lower, or delivery, end of the pipette is rinsed rapidly in saturated brine to remove debris, and then lowered carefully into the floatation fluid until this fluid also rises to the constriction (A). The floatation fluid thus pushes the faecal suspension upwards into the mixing bulb (B). With a finger over the upper end, the pipette is now lifted from the floatation fluid and tilted until its contents run into the mixing bulb. A gentle side to side rocking motion is used to mix the contents thoroughly. Samples are then transferred to the first and second chambers on the slide, the contents of the pipette being re-mixed before the second chamber is filled. The original watery faecal suspension is again mixed thoroughly and a fresh sample is withdrawn, mixed with the floatation fluid and transferred to the two remaining chambers.

2. *The Sliding-top Apparatus.*—Preparation of the watery faecal suspension, mixing, withdrawal of a sample and mixing with the floatation fluid is carried out as described above, but a larger pipette is used which delivers 2 ml. of the mixed faeces/K_2HgI_4 suspension.

The contents of the pipette are discharged into the trough as illustrated (Fig. 4). As the contents of the pipette run into the trough, the sliding top is pushed across to ensure complete filling without flooding or production of bubbles. After one minute the sliding top is pushed carefully back over the platform and carries with it the surface layer of suspension containing the trematode eggs.

There are some advantages in using this technique. The eggs are in a thin layer of fluid over a clear glass platform with no debris to interfere with the transmission of light, whereas the presence of much dark debris makes detection of the eggs more difficult in the counting slide. The area to be traversed in examining 2 ml. of material is reduced. Some eggs collapse and tend to sink, but they can be found easily by focussing on the platform.

3. *Counting the eggs.*—The eggs rise very rapidly and should be counted within five minutes of contact with the floatation fluid. The egg shells soon buckle and collapse. Counting is carried out by means of a mechanical stage. A 2/3 objective and a 5x eyepiece, or a lower-power objective and a 10x eyepiece, are used.

If a 4 gm. sample of faeces is taken, a factor of 50 is used for each chamber examined on the counting slide. When four chambers are examined (2 ml. of suspension) the factor is 12.5. It is derived as follows. The faecal suspension contains 4 gm. in 50 ml., the dilution with floatation fluid in the pipette reduces the proportions to 4 gm. in 100 ml. (1 gm. in 25 ml.); a total volume of 2 ml. is examined and represents 1/50 of the original suspension which contained 4 gm. faeces. Each egg counted represents 12.5 eggs per gramme in the original faecal sample.

When the sliding top apparatus is employed the whole 2 ml. of suspension is examined. It contains the eggs recovered from a 1.0 ml. sample of the original 4 gm. in 50 ml. water faecal suspension. The factor is again 12.5 for conversion of eggs counted to eggs per gramme of faeces.

DISCUSSION.

A series of comparative tests, made with faeces containing *Paramphistomum* eggs, indicated several sources of error.

A pipette designed to deliver exactly 2 ml. was tested, but the film remaining in the pipette, after delivery of the 2 ml., was found to retain some 10 per cent. of the eggs. Considerable care was also necessary to ensure that the contents of such a pipette were expelled as completely as possible.

The counting slide was satisfactory, provided there was adequate mixing before the filling of each chamber. If the first two chambers were filled from the pipette, without an intermediate mixing and likewise the remaining two chambers, a decrease was found in the number of eggs present in the second and fourth chambers. A corresponding increase occurred in the number of eggs remaining in the pipette.

The sliding-top apparatus was consistently accurate, provided the filling of the trough was carried out within a reasonable time (20–30 sec.), the number of eggs present in the fluid remaining in the pipette was at all times proportionate to the egg count in the apparatus.

SUMMARY.

A technique is described for the floatation and enumeration of trematode eggs. A special sampling and mixing pipette has been designed. The floatation fluid is a solution of potassium mercuri-iodide (K_2HgI_4). A modified McMaster egg-count slide, or else a calibrated sliding top-piece apparatus is used for counting the eggs and estimating the number per gramme of faeces. The apparatus is illustrated and described.

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On Treating and Preventing Gapeworm Disease.

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Syngamus trachea is one of the more difficult parasitic worms to dislodge owing to its position in the trachea. Many contrivances such as twisted feathers and spirals of fine wire have been used for the mechanical removal of the worms. In the hands of experts, they may be reasonably effective, but much injury frequently follows their use by amateurs, and the damage is often severe enough to cause the death of the bird. Anthelmintics which may be administered in food and water are seldom excreted by the lungs or are excreted in such low concentration as to be ineffective. Garlic oil and salicylic acid have been used, but are only very slightly effective and cannot therefore be considered as satisfactory treatments. Many so-called gapeworm cures consist largely of substances which stimulate the respiratory musculature and induce a sudden explosive cough. A few worms may be dislodged by this means, but far more are left, while the strain on the bird must be considerable and may indeed be more than a weakly one can stand.

Treatment.

Many authors have recommended fumigations and inhalations, and some of these are useful. The most recent one and certainly the most effective is the double tartrate of antimony and barium. This substance has undoubtedly a strong lethal action on the worms; they release their hold on the lining of the trachea and are coughed up within a few hours. But it also has a significant action on the epithelial membranes of the eye and orbit. Contamination of this region is followed by lachrymation and inflammation, which in mild cases clears up in a few days; in severe cases, however, the damage steadily progresses with ulceration followed by secondary bacterial invasion, which has in a number of experimental animals led to complete blindness. This condition resulted when adult hen pheasants were treated in a closed box, the substance being blown in upon them from above. The box was agitated to encourage dispersion of the powder. A later experiment was made with pheasant poults by dusting the breast feathers of the bantam foster mother at night. The poults nestled in among the feathers and inhaled the tartrate so that the worms were destroyed,

but a high proportion of the birds were blinded, as the ocular mucous membranes were in direct contact with the powder. Good results are, however, forthcoming among pheasants when the nostrils of sick birds are blocked with a little powder. This is gradually inhaled and a complete cure usually results within 24 hours. Only once among 82 experimental pheasants has a second administration been necessary, but the treatment has, so far, been less successful among partridges. This technique is simple, and takes only a few seconds to carry out; the powder being picked up on a finger tip and spread over the nostril. There is no danger to the eye. The disadvantage, as indeed with most useful gapeworm treatments, is that each bird must be handled separately, though this technique takes even less time than does dusting or any other individual procedure. It might not, however, be practicable where hundreds of birds are infested and it would be quite impossible to administer it to wild birds. Even when birds have been successfully treated, there is always the possibility—even the probability—that they will pick the infestation up again. The substance acts on gapeworm in partridge in exactly the same way, causing the buccal musculature to relax. But the partridge seems to have difficulty in coughing up the worms. Many natural infestations have been treated but in almost every case the worms have become packed into a solid mass which has completely obstructed the trachea and caused rapid death of the bird. When worms are not actually killed, they may also take anchor once again on the tracheal mucosa. It has not yet been possible to treat experimental infestations in the partridge as sufficient young stock has not been available.

Prevention.

Attempts have been made, therefore, to prevent infestation. Two lines of attack are possible. One is to sterilize the ground on which the birds are running. There are a number of chemicals which will destroy helminth ova and larvae, but they cannot generally be used over large areas because of the cost and because of various physical and mechanical difficulties. Furthermore, in the case of *Syngamus* it would also be necessary to destroy the wide invertebrate fauna which can act as reservoir and transport hosts of the infective larva. Effective worm eradicators are known but the fertility of agricultural land depends in part on the invertebrate fauna living within it, while terrestrial molluscs and the widespread Coleoptera are difficult to destroy under field conditions.

The second line of attack is the infective larva itself in the gut.

This appeared to be the weakest link in the life cycle, but the larvae only remain vulnerable so long as they are in the gut. As they have been found in the lungs as early as six hours after experimental feeding, it is clear that they can remain in the gut for only a very short time after hatching. Their journey to the lungs must begin immediately they are hatched. Any anthelmintic must therefore act rapidly and be fed continuously so as to be present whenever a larva is released by the digestive juices. This limits the choice considerably for many otherwise useful anthelmintics are toxic substances which can only be given under controlled conditions.

Phenothiazine is, however, comparatively bland in its action on an avian host, and there is a wide margin of safety between the clinical and lethal doses. Birds cannot, however, be induced to pick it up naturally even when it is flavoured with some attractive volatile oil such as aniseed. Chips of flavoured phenothiazine, the same size as gizzard grits, were placed in dishes before both partridges and pheasants ; they were immediately attracted to it, but by counting the chips and by weighing it was found that none had been taken. It was necessary, therefore, to incorporate it into the food so that the birds were compelled to take it.

A series of three pens known to be heavily seeded down with several helminth parasites, including *Syngamus trachea*, were used for some experiments. Young pheasant poults were chosen as experimental animals for they are readily infested with the available strain of the parasite, and they do not develop a strong degree of age immunity as do chickens. Birds were hatched in an incubator and reared on wire floors until 10 days old, after which they were introduced to these pens. The choice of birds for each pen was purely random, so that each contained a mixture of several varieties—melanistics, old English blacknecks, Chinese ringnecks and some hybrids. It may conveniently be remarked here that neither variety nor sex seems to have any bearing on the susceptibility of the young bird to infestation, and these factors therefore can be ignored.

Pen A was fed mash containing 4% phenothiazine.
Pen B „ „ „ „ 2% „
Pen C had pure mash and acted as controls.

Within 24 hours the birds in Pen A were noticeably darker than the others and began to show interesting eye symptoms which have already been described in a previous article. The birds in Pen B showed some darkening of the plumage and leg scales, but no eye symptoms. At six days some of the controls in Pen C began to look ruffled ; one died

the following day, and the lungs were found to contain 84 young *Syngamus* worms, some of which were coupled, while others were free. There was considerable exudate and haemorrhage. At 14 days two birds were definitely gaping—they died shortly afterwards and contained 15 and 11 pairs of worms respectively in the trachea. Within the next five days all the remaining control birds were gaping.

A much healthier state of affairs occurred in Pen B, fed 2% phenothiazine. A bird, No. 19, snicked after 18 days and continued to do so until it was destroyed at the end of the experiment, when it was found to harbour four pairs of worms. Within the next few days two others, Nos. 13 and 16, also snicked, but they too survived until they were destroyed at the end of the experiment. They carried three and five pairs of worms respectively. No ova could be found in faecal samples from the other birds, though they were examined twice weekly.

In Pen A all the birds remained perfectly healthy with no sign of disease. They were rather smaller than those of the other two groups, but were not lacking in vigour or energy.

All the birds were destroyed when 41 days old, i.e., 31 days after being exposed to infestation. Post mortem examinations gave the results summarized in Table 1.

These results were distinctly encouraging, and further trials were carried out on the same general lines. The same pens were used again, but this time the birds in Pen A were fed 1 per cent. phenothiazine, those in Pen B were the controls, while in Pen C, birds were fed 3 per cent. phenothiazine in the mash. Once again the experimental birds were chosen at random and were a mixture of several varieties and hybrids : there were also a number of different ages, as not enough of any one particular age was available. The results obtained are indicated in Table II. Survivors were destroyed 20 days after being placed exposed to infection.

It will be seen that 3 per cent. phenothiazine gives a very real protection against infestation, though a number of worms are able to slip through the defences and mature. There is, however, practically no protection from 1 per cent., a single bird remained free but the others in this group all developed appreciable numbers of worms and one died as a result. Control birds were all heavily infested, and several died.

Tests have also been carried out by infesting individual poults kept on wire floors with known numbers of infective ova. After a certain amount of trial and error, it was found that 200 ± 10 ova of this particular strain of gape worm, is a reasonable dose for pheasant poults aged 10

TABLE I.

Pen A. fed 4% phenothiazine			*Pen B fed 2% phenothiazine*			*Pen C Controls*		
No.	*Weight in Gm.*	*Gapes* Pairs	*No.*	*Weight in gm.*	*Gapes* Pairs	*No.*	*Weight in gm.*	*Gapes* Pairs
1	165	0	12	200	0	23	175	3
2	160	0	13	205	3	24	died	84 larvae in lungs
3	165	1 (very small)	14	195	0	25	215	8
4	200	0	15	180	2	26	died	15
5	165	0	16	200	5	27	230	2
6	235	0	17	185	0	28	210	9
7	165	0	18	195	0	29	250	3
8	205	0	19	195	4	30	died	11
9	170	0	20	205	0	31	215	20 (some young)
10	160	0	21	190	1 (very small)	32	230	13
11	195	0	22	195	0	33	250	22 (all young)
	Average 180.5	Total 1		Average 195	Total 15		Aver. 221.875	Total 106 + 84 larvae

days. Larger doses lead generally to lethal infestations, while smaller ones did not always give rise to a significant number of adults in the trachea.

Table II.

Pen	*Bird No.*	*Number of gapeworms*
A	65	died after 17 days—6 pairs
fed 1 per cent.	66	5 pairs
phenothiazine	67	5 pairs
	68	4 pairs
	69	0
	70	2 pairs
B	71	6 pairs
Control	72	died after 15 days—17 pairs
	73	9 pairs
	74	died after 6 days: many larvae in lungs
	75	5 pairs
	76	died after 7 days: many larvae in lungs
C	77	0
fed 3 per cent.	78	1 pair
phenothiazine	79	2 pairs
	80	0
	81	0
	82	1 pair

The birds to be infested were grouped as follows:—

Group A.—These birds were fed mash containing 3 per cent. phenothiazine which was given for the first time on the night before the

TABLE III.

Bird	*Pairs of worms*
A 1–6	0
B 1	0
2	0
3	2
4	1
5	2
6	5
C 1	6 (died after 10 days)
2	3
3	2
4	4
5	4
6	7 (died after 10 days)
D 1	10 (died after 8 days)
2	6 (died after 9 days)
3	8 (died after 9 days)
4	43 larvae in lung : (died after 5 days)
5	6
6	5
7	5
a 1	0
2	0
3	0
4	1
5	1
6	3
b 1	1
2	3
3	4
4	3
5	5 (died after 9 days)
6	2
c 1	4
2	8 (died after 8 days)
3	5
4	7 (died after 9 days)
5	3
6	5 (died after 10 days)
d 1	7 (died after 8 days)
2	9 (died after 7 days)
3	4
4	7 (died after 10 days)
5	5
6	6 (died after 12 days)

experimental feeding of ova. It was left in front of the birds for four hours afterwards, when ordinary mash was substituted.

Group B.—These birds were fed mash containing 3 per cent. phenothiazine, but it was not placed there until a few minutes before the experimental feeding occurred. It remained for six hours.

Group C.—Ordinary mash was given to the birds in this group until they had been fed the gapeworm ova. It was left for six hours.

Group D.—Controls, fed ordinary mash throughout the experiment.

These four groups were repeated with the sole difference that the experimental mash contained only 2 per cent. phenothiazine. In the table of results, this series is indicated by the lower case letters a, b, c and d.

Some of these birds died as a result of the infestation. Those which survived were destroyed 18 days after feeding. The results are given in Table III.

Conclusions.

Treatment of birds infested with *Syngamus trachea* is not an easy matter. Some considerable success has been achieved among pheasants with barium antimonyl tartrate, but partridges are less satisfactory subjects. Damage to the ocular membranes is prevented by manually blocking the external nares with the substance, which is then inhaled.

Phenothiazine is a useful substance for the prevention of infestation. Complete protection can be expected when it is fed in the mash at the rate of 4 per cent.: a very real degree of protection is given by mashes containing 3 per cent., while lower percentages are less useful. It is necessary to have the phenothiazine present when the larvae hatch, for they rapidly pass out of the intestine to safe positions in other organs where they are not readily attacked by chemicals. It is therefore necessary to feed the treated mash immediately before experimental infestations and continuously where the birds are always liable to pick up an infestation. This latter system may lead to some reduction in the weight of the bird which will be a distinct disadvantage to poultry farmers. Among pheasants, however, it is perhaps less important. The alternative will often be a dead bird, and even small pheasants are better than none at all. The danger period for infestation in poults is the first 10–12 weeks after which some natural resistance develops, though this not so complete as in poultry. Even if older birds become infested, they are generally able to withstand the serious effects and live, though as reservoir hosts they are a source of danger to other birds.

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Keratitis in Pheasants following Treatment with Phenothiazine.

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During the course of some anthelmintic tests carried out during June, 1949, it was necessary continuously to feed 4 per cent. phenothiazine to pheasant poults in the daily mash, which they ate without any obvious distaste and grew reasonably well. The birds were a mixture of several varieties of *Phasianus colchicus*, including some Formosan and Korean hybrids. These latter birds have a light coloured plumage and it was soon noticed that the feathers were darkening. The change was obvious less than 36 hours after the first experimental feeding. All the birds were therefore examined and it was found that all were similarly affected though it was less obvious among the melanistics and black-necks, which have a darker plumage. The leg scales too had taken on a distinct dark green hue, and this became more intense as the tests proceeded ; the pigmented feathers assumed a flat brownish colour, while the filoplumes are distinctly red. At the same time eye changes were noticed and their progress watched. At first there was increased secretion from the lachrymal glands, while the circumorbital tissues became oedematous. The cornea was cloudy and it rapidly thickened until it became opaque. These symptoms cleared up readily in a few birds that were removed indoors out of the direct sunlight. In two of the birds left outside they began to show signs of clearing, but in most cases they became more intense and it was followed in the most pronounced cases by ulceration and secondary bacterial invasion, when the eye became useless. The experiment had to be terminated after 32 days, by which time eight birds out of 12 were almost completely blind. Pigmentation of the feathers and scales was very marked but the actual skin seemed to be unaffected. There were no other symptoms. The birds fed well, grew and moved normally. There was no paralysis, muscular inco-ordination or stupor, and they showed no convulsive reactions to water.

It is known that some of the breakdown products of phenothiazine are excreted into the skin and its products, and there are records of sheep wool being dyed so intensely as to lose much of its economic value. Eye changes, too, have been noticed. Thorning noticed keratitis in pigs in 1942, as did Swales and his collaborators in the

same year. Britton made similar observations in 1943. Optical changes were reported in man by De Eds in 1940, while Whitten made close observation of the course of events in cattle in New Zealand. He carried out experiments among calves and was able to show that these changes only occur in bright sunny weather and never under cloudy conditions or among stock kept in houses.

It is worth noticing that this pheasant experiment took place in high summer with unusually long hours of bright sunshine, and the birds were confined in pens with little natural shade. Some cover was provided, but they preferred to run about in the sunlight. Recovery took place when they were removed from direct sunlight, provided the damage already done was not too serious.

Photosensitivity changes have also been noticed in cattle feeding on *Hypericum perforatum*, the main changes being seen in the skin and in a marked convulsive reaction when brought into contact with water.

All previous records have dealt with mammals, but it would appear that birds, too, can be sensitized by phenothiazine, even in a temperate climate, when there is plenty of bright sunshine.

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Snail Hosts of *Fasciola hepatica* in Britain.

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The liver-fluke, *Fasciola hepatica*, has a very wide geographical distribution and in various countries many different snails (usually species of *Limnaea*) have been identified as vectors. In Britain six native species of *Limnaea* have been described, yet, ever since Thomas (1883a) in England and Leuckart in Germany, established that *Limnaea truncatula* was a common vector, there has been little evidence that any other species of the genus serves as an intermediate host in Britain.

In view of a research programme on fascioliasis, which was planned at Weybridge under the direction of Dr. E. L. Taylor, it was clearly necessary to discover whether *L. truncatula* was the only possible host or whether other species of snail might prove suitable. Control of the snail seemed likely to prove most satisfactory for prevention of the disease and it was important to know if other vectors were concerned. While most authorities seemed to believe that *L. truncatula* was the only possible host in Britain, the evidence was by no means conclusive.

In his original investigations, Thomas (1883a) attempted to induce infection in fourteen different species of snail—including five species of *Limnaea* (*L. truncatula, L. stagnalis, L. pereger, L. palustris* and *L. auricularia*), but was unsuccessful except with *L. truncatula*. He (1883b) noted that the miracidia of *F. hepatica* penetrated *L. pereger*, while Leuckart in 1882 had stated that very young *L. palustris* were attacked. The parasite did not, however, continue to develop in either of these species. Following Thomas and Leuckart, various authors referred to the possibility of other species of *Limnaea* acting as vectors for *F. hepatica* in Britain. In 1917, Walton stated that he had found cercariae indistinguishable from those of *Distomum hepaticum* in a " sample " of *Limnaea pereger* which was obtained from a ditch containing numerous infected *L. truncatula* and in a letter to Nature (1923) he said that he had several times repeated the observation. In 1922 and 1923 Taylor, M., stated that she had on numerous occasions found examples of *L. pereger* harbouring perfectly developed cercariae of *Fasciola hepatica*. Unfortunately, neither of these authors gave full details of the observations and the evidence is not very conclusive. For example, Taylor (1922) describes finding free-swimming cercariae of *Fasciola hepatica* in material collected for protozoon studies. This seems most unusual for in our experience encystment occurs very

shortly after the parasites have left their snail host and it seems possible that Taylor was dealing with some other trematode species. Of the other Limnaeid species, *L. auricularia* was mentioned by the earliest workers as being resistant to infection, while *L. glabra*, although included in British lists from a very early date (da Costa, 1778) did not appear to have received attention from workers on *F. hepatica*. There was thus very little positive evidence of these Limnaeid snails acting as vectors for *F. hepatica*, but for two other species, *L. palustris* and *L. stagnalis*, there was strong evidence, since in both instances infection with *Fasciola hepatica* had been observed in Germany under laboratory conditions.

In 1924, Noller and Sprehn established cultures of *L. stagnalis* and used the young laboratory-bred snails for experimental infections with the miracidia of *F. hepatica*. About six weeks after the date of infection one snail, out of six examined, proved to be infected and cercariae from a crushed redia were seen, by the development of the cystogenous glands, to be approaching maturity. Similarly, Reichmuth (1936), in a paper which established the specificity of *L. palustris*, gave an account of an experiment in which he exposed 50 laboratory-bred *L. palustris* to infection with the miracidia of *F. hepatica*. Of 42 snails which survived for two to three months, 16 were found to contain cercariae and 11 contained rediae with daughter rediae. Full development and emergence of cercariae was not, however, observed.

There was thus no reliable record of the full development and emergence of cercariae from any snail known to occur in Britain except *Limnaea truncatula*. Nevertheless, there was a very strong presumption that both *L. stagnalis* and *L. palustris* could act as vectors and the possibility that some of the other species might become hosts under suitable conditions. This possibility has been investigated and the following account records laboratory observations concerning the susceptibility to infection of six British species of *Limnaea*. It will be shown that species other than *L. truncatula* are susceptible to infection and that development of the parasite within such snails may proceed as far as the emergence of fully developed cercariae, which encyst and subsequently can be shown to develop into mature flukes.

Material and Methods.

Snails used in the observations. When investigating the species which might serve as vectors for *Fasciola* it was decided to use only laboratory-bred snails. Preliminary observations had confirmed the work of Rees (1932), who found that a high percentage of wild Limnaeidae was

infected with a variety of trematode species which, especially in the early developmental stages, might be confused with *Fasciola hepatica*.

Furthermore, there was some evidence from laboratory investigation at Weybridge that molluscs already infected with trematodes acquired under field conditions might prove refractory to infection with *Fasciola hepatica*. Finally, the exclusive use of laboratory strains of the various snails obviated the difficulties of species identification encountered under field conditions, especially when dealing with the juveniles of these notoriously variable molluscan species. Accordingly, laboratory cultures of typical adult specimens of the various species of *Limnaea* were established while large numbers of susceptible *L. truncatula* were maintained for use as controls in the infection experiments. The method adopted for the culture of *L. truncatula* has been described by Taylor and Mozley (1948) and this was modified principally to suit the more aquatic members of the genus. All species seemed to appreciate a dietary supplement consisting of equal parts of powdered chalk and oatmeal, while some ate cabbage or lettuce leaves.

Methods of infection. Infection was carried out in Petri dishes or in 2½ litre Kilner jars, by exposing the snails to the mass attack of large numbers of active miracidia which had recently hatched from clean cultures of *Fasciola* eggs, isolated from gall-bladders of infected mammals and incubated at the laboratory. The infectivity of the miracidia was proved by their effect on *L. truncatula*, Weybridge snails being uniformly susceptible.

The methods used in the investigation were similar for all the species and since the successful development of the parasite was first observed in *L. stagnalis* (Kendall, 1949a), observations on this species will be considered in some detail.

Experimental Infection of *L. stagnalis* with *F. hepatica*.

Preliminary observations showed that, while the miracidia of *F. hepatica* attacked *L. stagnalis* of all sizes, infection did not persist in the older and larger snails: rediae developed only in the smallest individuals of each group. An experimental infection was therefore arranged in which a large number of freshly-hatched *L. stagnalis* was used.

Egg-masses laid by a laboratory strain of *L. stagnalis* were placed in a Kilner jar containing sterilised mud and filtered pond water. After seventeen days recently hatched snails were noticed in the jar and a culture of the eggs and freshly-hatched miracidia of *F. hepatica* was added. Snails already hatched would thus be exposed to the attack

of active miracidia while more miracidia might be expected to hatch over a period of days. In order to ensure that all recently hatched snails were exposed to the attack of fully active parasites, a further culture of eggs and miracidia was added eighteen days later. Twenty-five snails from this group were dissected after intervals of 41–67 days from the date of first infection and of these, three were found to contain the rediae of *F. hepatica*.

As the observation continued a substantial mortality occurred among the remaining snails but of 76 which were examined 80 days after infection ten were found to be infected and these contained rediae up to 1.5 mm. in length together with cercariae which appeared mature. Three infected snails (in which parasites could be observed through the transparent shell) were kept alive until cercariae emerged. Of these three snails, the largest measured 0.82 cms. in length and rediae, with their contained cercariae, could be seen in the body cavity, lying between the crop and the digestive gland. A single cercaria actually emerged 87 days after the date of infection and 26 days later 86 cercariae left the snail during a 24 hours period, encysting on the walls of the vessel and on a piece of cabbage which had been supplied as food for the snail. These cercariae were indistinguishable from those of *F. hepatica*. In all, 99 perfect cercariae emerged from this snail 3–4 months after infection. Four parasites were placed in the stomach of a rabbit and in six weeks' time a half-grown specimen of *F. hepatica* was recovered from the bile-ducts, thus demonstrating conclusively that the cercariae which had emerged from the *L. stagnalis* were in fact those of *F. hepatica*.

At the time of emergence the snail measured 1.01 cms. in length and was about four months old. Three weeks later it had grown to a length of 1.32 cms. showing all the characteristics of a typical British *Limnaea stagnalis*. By this time, the shell had darkened so that infection could no longer be determined by examination of the live snail, which continued its normal growth and finally produced four fertile egg-masses. The snail was then dissected but was found to be free from parasites and there was no macroscopic sign of injury to the digestive gland.

In this way it was clearly demonstrated that *F. hepatica* could complete its intramolluscan development in *L. stagnalis*, for out of 101 snails which had been exposed to infection, thirteen were shown to contain the parasite and from one snail numerous fully mature cercariae emerged.

EXPERIMENTAL INFECTION OF *L. palustris*.

Observations showed that *L. palustris* also was susceptible to infection with *F. hepatica* and preliminary experiments confirmed the work of Reichmuth (1936) who demonstrated that development progressed as far as the formation of rediae and cercariae. To extend this observation, egg-masses from a pure culture of *L. palustris* were allowed to incubate in a Petri dish containing a little filtered pond water. As soon as hatching occurred, eggs and freshly hatched miracidia of *F. hepatica* were added. After eight days, the infection was repeated in an effort to ensure that all newly-hatched snails were exposed to the attack of fully active miracidia. About six weeks after the date of infection, two snails were dissected, one (measuring 0.50 cms. in length) proving negative while the other (0.48 cms. in length) contained rediae and first-stage cercariae. During the following two months 101 snails were examined and of these, 34 were found to contain well-developed infections of *F. hepatica*. Three months after infection, cercariae were known to have emerged from each of twelve snails which eventually developed into typical *L. palustris* measuring up to 1.50 cms. in length. Cercariae which emerged from these snails proved infective for mice and developed into typical young *F. hepatica*.

EXPERIMENTAL INFECTION OF *L. glabra*.

Newly hatched snails of this species were exposed to the mass attack of fully active miracidia. About two months after infection 46 snails were examined and of these, eight contained mature or nearly mature cercariae which were observed through the transparent shells of the young snails. One month later, mature cercariae emerged from five of the snails, as many as 156 leaving a single snail during a period of 24 hours. These cercariae proved infective for mice and young liver flukes were found on post-mortem examination 21 days later.

EXPERIMENTAL INFECTION OF *L. peregeri*.

Preliminary observations showed that juveniles of this species were attacked by the miracidia of *F. hepatica*. In some snails, sporocysts developed and during the first six days the growth of the parasite was comparable with that which occurred in *L. truncatula*. Further development was observed on one occasion only ; out of a group of 108 snails, a single specimen contained six rediae in some of which were immature cercariae. In a further group of 88 snails which had been exposed to infection immediately after hatching no sign of parasitism was evident

when the snails were examined two months after infection had been carried out.

Experimental Infection of *L. auricularia*.

Young laboratory-hatched *L. auricularia* were exposed to mass infection with *F. hepatica* under similar experimental conditions to those adopted for the other species. Snails dissected about six weeks after the date of infection showed no signs of parasitism. Altogether 87 snails were dissected at intervals until three months after infection, when the observation was discontinued. No sign of infection was noted in any of the snails examined.

Further Observations on Experimental Infections.

It has been shown that freshly-hatched *L. palustris*, *L. stagnalis* and *L. glabra* are susceptible to infection with *F. hepatica*. Under the experimental conditions *L. pereger* and *L. auricularia* proved refractory but it is quite possible that some variation in the technique of infection might show that they also are susceptible and that the parasite may develop to maturity. Some further observations made during the experimental infections will now be considered.

Variation in susceptibility to infection with F. hepatica. *L. truncatula* differs markedly from all the other British species of *Limnaea* in its relationship to *F. hepatica*. It seems unique in being susceptible to infection at all ages and in all stages of growth. Failures to infect snails of this species at the Laboratory are practically unknown. On the other hand, infection of the other species appeared to occur only with the very youngest individuals. Furthermore, only a proportion of each newly-hatched group became infected, thus indicating that there was a variation in susceptibility even between snails apparently uniform in age and size. Thus, only about 13% of one uniform group of *L. stagnalis* were found to contain the parasite whereas every *L. truncatula* in a similar group became infected.

The resistance to infection of these species of *Limnaea* is evidently relative and not absolute and it is important to consider what causes the variation in susceptibility. Two possibilities were investigated under laboratory conditions. Firstly, it was considered possible that the young snails used in the experiments might differ genetically in their susceptibility to infection. Secondly, there might be some similar inherent variation in the miracidia obtained from bulked samples of the eggs of *Fasciola hepatica*. It was decided to carry out some

preliminary observations which might show the existence of biological races of *L. stagnalis* or of *F. hepatica.*

Observations on a susceptible strain of L. stagnalis. In an attempt to demonstrate a higher rate of infectivity in a susceptible strain of *L. stagnalis*, young snails were reared from the self-fertilisation of a snail which was known to have been infected with *F. hepatica* and from which numerous mature cercariae had emerged. Two groups of the progeny were exposed to infection with the miracidia of *F. hepatica.* One group was infected, immediately after hatching, in a Petri dish containing very large numbers of active miracidia. After 24 hours all unhatched snail eggs were removed. Under such conditions a high rate of infectivity was expected and in fact out of 63 snails examined 23 were shown to have developed mature infections of *F. hepatica.* The second group was exposed to infection in a Kilner jar, eight days after hatching had been observed. Not a single infected snail was found during the dissection of 33 of the group, thus suggesting that the age of the snails and the conditions of the experiment were the important factors in inducing infection.

It is apparent that further work on the existence of a biological race of *L. stagnalis* is needed, but in the series of observations described there was no evidence that the group was particularly susceptible owing to its origin from a snail which had itself proved susceptible to infection.

In practice the highest percentage of infected snails was obtained when an egg-mass in which hatching was just commencing was exposed to large numbers of active miracidia.

Observations on cercariae which emerged from L. stagnalis. Cercariae which emerged from a *L. stagnalis* which had been experimentally infected with *F. hepatica* encysted normally and proved infective for laboratory rabbits. Several of these rabbits were killed after a suitable interval and large numbers of the eggs of *F. hepatica* were recovered from the gall-bladders. Miracidia which resulted from the incubation of these eggs were used in an attempt to infect a group of *L. stagnalis* which measured between 0.58 cms. and 1.08 cms. in length and which, since they were known to have been hatched several months previously, would not ordinarily prove susceptible to infection with *F. hepatica.*

In fact, of 31 snails examined, none appeared to have become infected, whereas each snail in a control group of *L. truncatula* became infected with the parasite. There was, therefore, no evidence of the existence of a biological race of *F. hepatica* to which *L. stagnalis* was particularly susceptible.

Pathogenicity of F. hepatica *for the various* Limnaea *species.* In

general, the course of the infection in other species of *Limnaea* was parallel to that observed in *L. truncatula*. As far as could be observed, infection took place under similar conditions, primary sporocysts became established in the mantle region, young rediae could be found along the intestinal tract and in the digestive gland, cercariae matured in the body cavity and the rate at which the various parasitic stages developed seemed to be the same in all species. In one important particular, however, the development of the parasite in *L. truncatula* differed from that in the other species. While, as will be shown elsewhere, *F. hepatica* is to some extent pathogenic for *L. truncatula*, snails kept under good environmental conditions and not exposed to unduly high temperatures do not appear to suffer and growth is not retarded. With the other species, however, the parasite appears to have a marked pathogenic effect, growth is severely retarded, shells are abnormally transparent and lacking in pigmentation, shell whorls are distorted and the snails themselves appear lethargic, obviously suffering severely from the effects of the parasite. In the laboratory such snails were maintained alive with difficulty although when kept separately in small culture dishes and supplied with ample quantities of readily available food, the rate of growth improved. Return to a normal appearance with improvement in shell texture and colour apparently coincided with loss of infection.

TABLE I.

Comparing the mean shell lengths of infected and uninfected snails of four species of Limnaea.

Species	*Infected Snails*			*Uninfected Snails*		
	Number	*Mean Shell Length*	*Standard Error*	*Number*	*Mean Shell Length*	*Standard Error*
L. truncatula	25	0.71 cms.	±0.020	25	0.69 cms.	±0.017
L. palustris	34	0.33 cms.	±0.014	78	0.63 cms.	±0.0061
L. glabra	8	0.39 cms.	±0.014	38	0.86 cms.	±0.012
L. stagnalis	20	0.63 cms.	±0.010	20	1.00 cms.	±0.0085

Table 1 illustrates one aspect of the pathological effect of *F. hepatica* on some species of *Limnaea*. Groups of snails of the species

L. palustris, *L. glabra* and *L. stagnalis* were exposed to infection with *F. hepatica*. After a suitable interval, the snails were examined and differentiated into those with established infections and those which appeared uninfected. To compare the growth of *L. truncatula* a uniform group of snails was divided, half being exposed to infection while the others were kept as controls. Mean shell lengths (with standard errors of the means) are tabulated. It will be seen that a highly significant difference exists between infected and uninfected snails of the three species *L. palustris*, *L. stagnalis* and *L. glabra*, whereas there is no such difference between the two groups of *L. truncatula*. It is believed that the significant differences between infected and uninfected snails is a result of the infection, although the observation would be explained equally well if initially weakly snails, without much potentiality for growth, were differentially infected.

Loss of infection in species of Limnaea. Cort (1941) describing observations on Limnaeidae, Physidae and Planorbidae which had been infected with digenetic trematodes, stated that in all his examinations he saw no evidence suggesting recovery from an old infection. Extensive observations on *L. truncatula* at Weybridge have suggested the same conclusion, since snails which have been kept in full activity for periods of as long as a year (probably the maximum expectation of life under laboratory conditions) have remained infected until death.

It is interesting to note, therefore, that loss of infection was observed with another British species of *Limnaea*. Thus a *L. stagnalis*, from which over 100 cercariae of *F. hepatica* had emerged, was kept at the laboratory for a period of eleven months after the date of infection. When dissected, no evidence of the parasite was seen. Observations on a further small group of the same species suggested that infection was maintained for about three months, when only a very few undeveloped rediae could be found in snails which had previously contained large numbers of mature cercariae.

The numbers of parasites becoming established in individual snails. Our observations showed that many more parasites became established in *L. truncatula* than in other susceptible species, this being associated, perhaps, with the fact that many more sporocysts developed in *L. truncatula* than in the other species of *Limnaea*. It was rare to find more than one sporocyst in a young *L. palustris* examined soon after infection, whereas in a *L. truncatula*, infected at approximately the same age and under the same conditions, as many as 30 developing sporocysts might be demonstrated. Dissection of infected *L. palustris* and *L. glabra* at more advanced stages in the development of the

parasite suggested that not more than 30 rediae had developed, as compared with the more usual 150–300 found in *L. truncatula* infected under similar conditions. Kendall (1946b) working with *L. truncatula* and *F. hepatica*, showed that the development of such a small number of rediae was usually related to infection with a single miracidium. In species other than *L. truncatula* the development of small numbers of rediae must be referred to the general resistance of the snail to infection with *F. hepatica*.

Discussion.

From these observations it is apparent that *L. truncatula* is much more susceptible to infection with *F. hepatica* than are the other British species of *Limnaea*.

As a host of *F. hepatica*, the snail *L. truncatula* appears to be particularly well adapted both in its uniform susceptibility to infection and, as shown by Kendall (1949b) in the way in which the rate of growth of the parasites is adjusted to accommodate the host during times of starvation or drought-induced aestivation. In view of the excellent adaptation which appears to exist between *L. truncatula* and *F. hepatica* it is reasonable to conclude that the snail rarely suffers from retarded growth or the abnormal development which appears to characterise infected snails of those Limnaeid species in which the fluke is an aberrant parasite.

Reference of the observations to the control of Fascioliasis as a disease of domestic animals.

It must be concluded that in Britain *L. truncatula* is the normal host of *F. hepatica* and that development of the parasite in other species is limited by their relative resistance to infection and by the pathogenicity of the parasite.

From theoretical considerations, it is not likely that any considerable proportion of a field population of species other than *L. truncatula* will be found to contain mature infections of *F. hepatica*. Under laboratory conditions *L. truncatula* is uniformly susceptible to attack while the other species of *Limnaea* are partially susceptible for a very limited period after hatching. In the field, Rees (1932) found that only about 7% of *L. truncatula* were infected and our field observations have confirmed that, while there is great local variation, not more than 5% or 6% of adult snails of this species are infected. If the highly susceptible species *L. truncatula* is normally parasitised to such a small extent under field conditions it is not likely that large numbers of the other species of *Limnaea* will be found to be infected.

In addition, ecological evidence suggests *L. truncatula* as a most efficient vector, for it is usually confined to shallow temporary water and to muddy ground where frequent alteration of drought and flooding leads to conditions optimum both for infection of the snail and for the infection of grazing stock. The other British species of *Limnaea*, with the possible exception of *L. glabra*, are more aquatic in habit and any snail species living in a large volume of permanent water is far less likely to become infected during the limited life of the miracidium of *F. hepatica*, while cercariae released under such conditions are unlikely to come within the grazing range of domestic stock or of wild rabbits.

In conclusion, it does not appear that species other than *L. truncatula* play an important part as vectors of *F. hepatica* in Britain. Rees (1932) failed to find any sign of *F. hepatica* in 1046 *L. stagnalis*, 1332 *L. pereger* and 219 *L. palustris* which she collected in the field, but it would nevertheless be of interest to carry out a really extensive survey of field populations of the various species of *Limnaea*, particular attention being paid to small and ill-developed snails, which laboratory experience has shown most likely to harbour the parasite.

It is frequently difficult to ascertain the presence of *L. truncatula* in the field owing to its occupation of temporary pools and its aestivation under dry weather conditions ; hence it is important not to be too hasty in assuming the presence of other vectors in areas where *F. hepatica* is known to occur in stock, but where *L. truncatula* cannot easily be demonstrated. There is no doubt that *L. truncatula* acts as host in the great majority of instances.

SUMMARY.

1. This paper presents experimental evidence to show that *Limnaea truncatula* is not the only British snail susceptible to infection with *Fasciola hepatica*.

2. Of the six British species of *Limnaea*, five may be infected under laboratory conditions, *L. auricularia* proving resistant to infection. Full development of the parasite occurs in *L. truncatula*, *L. stagnalis*, *L. palustris* and *L. glabra*, while development as far as the production of rediae occurred on one occasion in *L. pereger*. Of the susceptible species *L. truncatula* may be infected at any age or size, but the other species are susceptible only during the first few days after hatching.

3. *F. hepatica* appeared to be relatively pathogenic to hosts other than *L. truncatula* even though the level of infection (as evidenced by the number of rediae which developed) was considerably lower than in the more usual host.

4. It is concluded that *L. truncatula* is the only important vector of *F. hepatica* in Britain, although other species of *Limnaea* may be expected to act as occasional hosts under field conditions.

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Investigations on the Emergence of Larvae from Cysts of the Potato-Root Eelworm *Heterodera rostochiensis.*

2. The Form of the Hatching Curve.

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In a previous paper (Fenwick, 1949), the author dealt with the sources of variation in the number of larvae emerging from cysts of the potato-root eelworm, *Heterodera rostochiensis* immersed in potato-root diffusate. No attention was paid in that paper to the rate of emergence nor to the form of the hatching curve. It is well known that the rate of hatching is not constant during the period of immersion in diffusate ; moreover, if during the course of hatching the diffusate is removed and replaced by a fresh sample from another source, substantial differences in the rate of hatching may occur as a result of the change. When cysts are immersed in a diffusate which is periodically replaced by fresh material from the same bulk source, it is generally found that after an initial period of little or no activity, a few larvae begin to emerge, the rate of emergence increasing gradually to a maximum and then tailing off slowly to zero. The whole cycle may take any time up to two or three months. While this general picture of larval emergence is probably well known, little or nothing is known regarding the form to which the hatching curve approximates ; as a consequence it is not possible to make any studies of known accuracy on the effect of different factors on hatching time, nor is it possible, knowing the number of larvae hatching over a period, to forecast what the total hatch will ultimately be.

Considering the hatching process from first principles, it would seem that the emergence of a larva from a cyst is the culmination of a process involving three stages : the penetration of the diffusate through the cyst-wall and subsequently the egg shell ; the stimulation of the contained larva to break out of the egg shell ; lastly, the movement of the larva through the cyst contents and its final emergence from the cyst. The first of these being a physical process of diffusion would not be likely to affect hatching to any great extent once penetration

through the egg shell was complete. The second stage being biological, would be expected to conform to some biological law and it is likely that this stage is the limiting factor in the whole process. The third and last stage, emergence of the larva from the cyst must obviously be subject to wide variations due to differences in the degree of fulness of cysts, competition with other larvae for egress, etc. One would, therefore, expect that this stage in the hatching process would introduce errors into any theoretical curve representing the emergence of larvae. If, therefore, the theoretical form to which the hatching curve approximates were found, then frequent and substantial departures from it would be likely to occur. It would, nevertheless, be useful to obtain information concerning this theoretical form in order to make possible new quantitative studies of the hatching process.

The most usual forms of variability are those which approximate to the normal distribution, and it would appear likely that the time taken for individual larvae to react to root diffusate (or some normalising function of times) should approximate to normality. The treatment of response data where this relationship holds has been formulated by Bliss (1937), who shows that where susceptibility, expressed as a function of either concentration or time, is normally distributed, it is possible to express the relation between response and dosage in a linear manner. This is accomplished by the use of probits which are a function of the percentage of individuals responding to a given treatment; the response, expressed in probits, being plotted against some suitable function of time of exposure; the resulting curve should then approximate to a straight line. The present paper seeks to demonstrate that this normal relationship does in fact apply to the time-susceptibilities of larvae to root diffusate, provided that a suitable function of time is chosen, and also to demonstrate the form of the appropriate function.

Technique.

The collection and storage of root diffusate has been previously described (Fenwick, 1949) as well as the technique for single cyst hatching. Experiments using samples of 100 cysts result in the production of large numbers of larvae and recourse has to be made to a sampling technique for their enumeration. The process of multiple hatching is carried out in solid watchglasses sometimes known as embryo dishes, approximately 1¼ in. square. The selection of a sample of 100 cysts is dealt with in a paper at present in course of publication.

The selected sample of cysts is placed in a watchglass and water added ; after soaking for a matter of three weeks the water is removed and replaced by fresh diffusate. The larvae liberated are removed at regular intervals and transferred to a 3 in. × 1 in. tube to which is added a little formalin to act as a preservative. The dilution technique is described in the paper in course of publication already referred to.

Procedure and Results.

Before considering procedure and results it might be advantageous to set out assumptions on which the experiments are based. It is assumed that when replicate batches of cysts are exposed to the action of a given sample of diffusate under fixed conditions, the number of larvae that will hatch from those cysts is constant apart from random fluctuations from sample to sample due to the inherent variability of the materials. These " hatchable " larvae are considered to represent those individuals present which react to diffusate and are therefore taken to represent the number of individuals exposed to treatment. Larvae left behind in the cysts after termination of hatching are ignored in these experiments, and when percentages or probits are referred to, they are calculated from the total number of larvae which emerged from the cysts.

Preliminary experiments were conducted in which cysts were hatched in potato-root diffusate, the number of larvae emerging at the end of definite times being recorded. The experiment was continued until hatching had completely ceased. The cumulative hatch at the end of each period of time was then expressed as a percentage of the total number of larvae emerging by the end of the experiment. Reference to a table of probits then enabled the percentage to be converted to a probit value and plotted against time. A curve with a downward concavity resulted from this treatment of the data ; a plot of probits against the logarithm of time resulted in an apparently straight line whilst plotting against the reciprocal of time resulted in a curve with an upward concavity. This preliminary result was confirmed repeatedly with a variety of *Heterodera* material, and it was therefore concluded that in all probability, the most promising line of approach was to assume that log-time was the most suitable function of susceptibility for future investigation.

To test this conclusion experiments were conducted on individual cysts, using the ordinary single cyst technique. The cysts used for this experiment were drawn from no less than four different batches. To

obtain more or less evenly spaced points on a log-time scale, the liberated larvae were counted at the end of 2, 4, 9, 16, 40 and 60 days. The cumulative hatches at the end of each time were then converted to probits and plotted against log-time. Considerations of space preclude the publication of all the graphs obtained but those shown in Fig. 1 are obtained from the first 10 results. It will be seen that agreement with a straight line relationship is tolerably good. In each graph the straight line drawn through the points is a line computed from the data. Analyses were then carried out to test the degree of agreement

TABLE I.

Analysis of Single Cyst Hatching Data.

No.	N	$\bar{x}$	σ	g_1	$^t g_1$	g_2	$^t g_2$
1	329	0.649	0.195	—0.155	—1.08	—0.0075	—0.056
2	37	0.919	0.423	—0.492	—1.32	—0.613	—0.806
3	100	0.695	0.264	+0.339	+1.39	—0.440	—0.960
4	161	0.682	0.157	+0.340	+1.75	+2.480	+6.430
5	25	0.726	0.303	—0.413	—0.628	+0.106	+0.117
6	68	0.590	0.300	+0.073	+0.25	+2.46	+4.1
7	727	0.550	0.214	—0.450	—4.95	+0.006	+0.024
8	398	0.577	0.240	+0.116	+0.94	+3.240	+1.850
9	188	0.607	0.258	+0.034	+0.19	—0.029	—0.081
10	191	0.680	0.215	+0.239	+1.35	—0.910	—2.53

between the computed line and the points. The theory of the analysis is adequately dealt with by Bliss (1937) and little point is served by describing the method here; suffice it to say that two statistics are computed, together with their standard errors; g_1 which measures the symmetry of the alleged standard curve from which the data is derived and g_2 which measures kurtosis. In a normal curve both these statistics should approximate to zero, i.e., they should not be significantly greater than their own standard error as disclosed by a t test. A summary of the results is set out in Table I. This table gives the

salient parameters necessary for drawing the computed line as well as the magnitude and sign of g_1 and g_2 together with their t values. It will be seen from this table that generally speaking the value of t

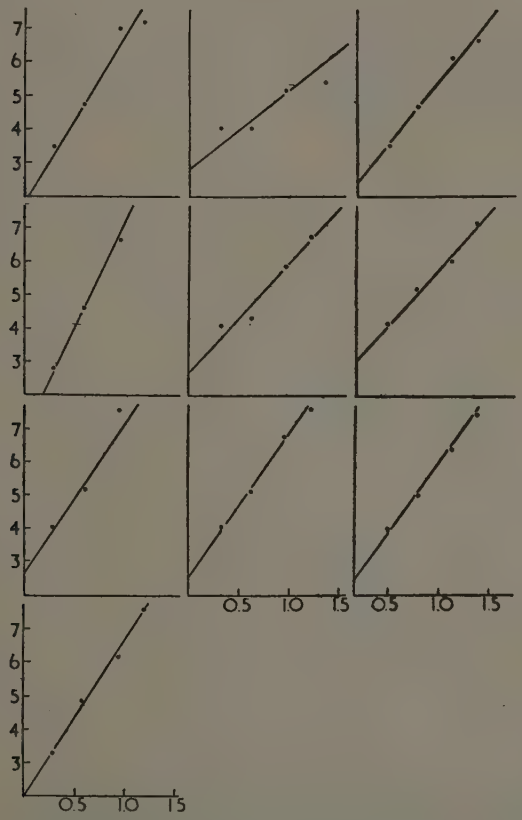

Fig. 1. Probit lines plotted for the hatch from 10 individual cysts in potato-root diffusate. Probits plotted vertically, log-time horizontally.

for g_1 is below the significance level except in the case of cyst no. 7 where an anomalous value of 4.95 is obtained. It will be seen moreover that six values of g_1 are positive and that four values are negative which

discounts any systematic tendency to asymmetry. Study of the values of t for g_2 discloses three significant values ; cysts number 4, 6 and 10 with values of +6.43, +4.1 and —2.53 respectively. The value of +1.85 for cyst number 8 is nearly significant, the other values of t are well within the significant limits. It is therefore reasonable to assume as a working approximation that susceptibility of larvae to the action of root diffusate is normally distributed within single cysts, when susceptibility is expressed as log-time.

A further experiment was conducted in which batches of 100 cysts

TABLE II.

Analysis of Multiple Cyst Hatching Data.

No.	*N*	$\bar{X}$	σ	g_1	t_{g_1}	g_2	t_{g_2}
1	82	0.656	0.282	+0.056	+0.211	—0.347	—0.66
2	134	0.668	0.287	+0.468	+2.2	+0.179	+0.09
3	101	0.626	0.360	+0.602	+2.52	—0.201	—0.41
4	340	0.656	0.300	+0.154	+1.195	+0.075	+2.35
5	173	0.572	0.300	+0.572	+3.05	+0.177	+0.46
6	137	0.538	0.348	+0.356	+1.70	—1.150	—2.74
7	77	0.525	0.297	+0.343	+1.25	+0.925	+1.71
8	172	0.542	0.385	+0.635	+3.40	+0.087	+0.23
9	130	0.576	0.320	+0.865	+4.05	+0.215	+0.50
10	1,163	0.565	0.294	+0.235	+0.31	+0.535	+3.68

were exposed to the action of potato-root diffusate. Larvae were removed at 2, 4, 8, 16, 40 and 60 days after immersion. The larvae were enumerated by dilution, the degree of dilution being kept constant throughout the experiment. The count thus obtained was therefore a constant and fixed fraction of the number of larvae emerging in a particular interval ; data from this experiment are therefore subject to a new error which does not apply to the data for single cysts, viz., a dilution or Poisson error. One cannot expect therefore that agreement with the theoretical line should be as close as in the former case. The

result of plotting probits of total hatch against log-time are presented in Fig. 2 from which it will be seen that agreement with the computed line is good. A summary of data appertaining to the curves is set out

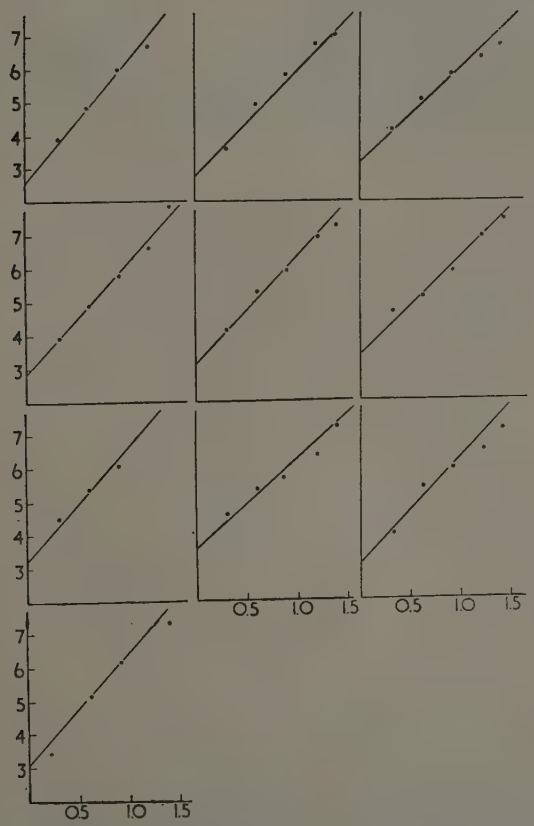

Fig. 2. Probit lines plotted for the hatch from 10 batches of 100 cysts in potato-root diffusate. Probits plotted vertically, log-time horizontally.

in Table II. It will be seen that theoretical agreement is not nearly so good for this set of data as for single cysts ; there is a marked tendency for g_1 to assume a positive value, significance being reached in this

direction no less than six times in the set of ten curves. There are three significant values for g_2 but in this case there is no evidence of any systematic deviation from zero, since both positive and negative values are recorded for this parameter.

The occurrence of significant and positive values for g_1 indicates that the distribution of susceptibilities is to a degree asymmetric, there being an excess of individuals whose susceptibilities are lower than the mean. This deviation could result from a number of causes. If there were mechanical damage to some of the cysts in a batch, they would

TABLE III.

Method for plotting time probit curves for larval emergence.

Class units in days	*No. of larvae hatching in each interval*	*Larvae hatched out end of each interval*			*Log of class units*
		No.	*%*	*Probit*	
0					
2	20	20	6.1	3.45	0.301
3	42	62	18.5	4.10	0.477
4	61	123	37.3	4.68	0.602
6	123	246	74.6	5.66	0.778
9	76	322	97.7	7.00	0.954
16	2	324	98.4	7.14	1.204
40	5	329	100	—	1.602

hatch more rapidly than the rest and would therefore finish sooner, the result of this would be an increased number of larvae hatching early in the experiment, producing a skew distribution. Study of the graphs of Fig. 2 would indicate however that this effect is not sufficient to affect the form of the probit curve to any great extent. It is, therefore, considered that for practical purposes it can be ignored and a linear probit relationship to log-time is a sufficiently close approximation to the actual form of the hatching curve.

Implications of the Probit Relationship.

Having established the probit relationship for the hatching curve of larvae of *Heterodera rostochiensis*, it remains to interpret its implications regarding the study of the parasite. When different batches of cysts are exposed to the action of several samples of root diffusate, a series of curves will be obtained when hatch is plotted against time. Since these curves will obey the probit relationship, they can differ from each other in three respects ; the total number of larvae emerging by the time hatching has finished, dictating the final height of the curve ;

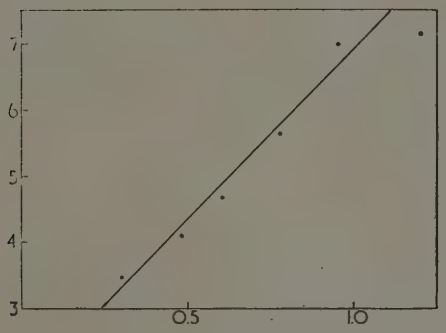

Fig. 3. Data from Table 3 plotted as probit x log time. Probits vertically, log-time horizontally.

the average time taken by the larvae to emerge from the cysts governing the position of the curve along the time axis ; and the variations shown by larvae about the mean hatching time, which determines the interval along the time axis which the curve occupies. These three parameters are sufficient to determine and define any particular curve and enable complete comparisons to be made between different curves.

The estimation of the total number of larvae emerging is a simple matter and is fully dealt with in another paper. The other two parameters are obtained from the probit line. Being a straight line, this can only vary in two ways—position and slope ; these two correspond to the two parameters of the hatching curve. The position

of the probit line is governed by the " mean hatching time " and is the value of log-time corresponding to a probit of 5.0. This can be read off directly from the probit line. Should greater accuracy be desired, it can be calculated, and its own standard error estimated. It corresponds to the log of the time at which 50 per cent. of the larvae have emerged and therefore the geometric mean time taken by the larvae to hatch.

The slope of the probit line corresponds to the standard deviation of the mean hatching time ; it is the amount by which log-time is increased when there is a rise of one in the probit value. It can be read off from the probit line or, as in the case of the mean, can be calculated from the data, together with its own standard error. Twice this value added to, and subtracted from, the mean, will give the log of time limits between which 95 per cent. of the larvae emerge from the cysts. Statistical comparison thus becomes possible between all three components of several sets of hatching data and renders it possible to determine the exact effect of different treatments or diffusates on hatching.

The Plotting of Time-Probit Lines.

For the benefit of workers who might wish to investigate hatching phenomena but who do not wish to enter into the statistics of the method, it is felt that a useful purpose might be served by including at this stage a short description of the method of plotting the probit regression line. Table III gives data resulting from a hatching test on a single cyst immersed in potato-root diffusate. Column 1 shows the time in days when the number of larvae were counted, the number of larvae liberated in that particular interval being recorded in column 2. These are then added up to give cumulative hatches as shown in column 3. The cumulants are then expressed as percentages of the grand total, 329, found at the foot of this column and are then entered in column 4. Conversion of the percentages to probits is accomplished by reference to a Table of Probits which may be found in Bliss's original paper (1935) or in any standard set of Statistical Tables (Fisher and Yates, 1938). The probit value is entered in column 5. The last column is merely the logarithm of column 1. A graph is now drawn in which the probit values are plotted vertically against the log class limits horizontally. Fig. 3 gives the graph resulting from such a plot. The value of x for a probit value of 5.0 is seen to be 0.63 and this is the logarithm of the time in days at which 50 per cent. of the larvae

emerge, i.e., the mean hatching time. Reference to a table of common logarithms shows that this corresponds to a value of 4.266 days. Further study of the line indicates that for an increase of 1.0 in the probit value from 5.0 to 6.0 the x or log-time value rises from 0.63 to 0.82, an increase of 0.19, which thus becomes a logarithmic measure of the variability in the hatching time. In this way the three parameters fixing the height, position and shape of the original hatching curve are estimated.

Summary and Conclusions.

Experiments were conducted in which single cysts and batches of 100 cysts were exposed to the action of potato-root diffusate and the number of larvae liberated at the end of certain intervals of time recorded. Investigation showed that when the hatch at the end of each time interval was expressed as a probit of the total hatch, and plotted against the logarithm of that time, a close approximation to linearity resulted. Statistical analysis confirmed this agreement in the case of single cyst hatching, but disclosed a significant degree of asymmetry in the case of multiple hatching. It was decided that the degree of curvature resulting from this when plotted as a probit line was sufficiently small for it to be ignored.

The implications of the probit relationship are discussed and its application to the examination of hatching data explained. It is concluded that probit analysis in conjunction with the total number of larvae liberated is sufficient to enable complete comparisons to be made between different sets of hatching data.

A description is given of the method for plotting the probit line visually and estimating its parameters.

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Investigations on the Emergence of Larvae from Cysts of the Potato-Root Eelworm *Heterodera rostochiensis*.

3. Larval Emergence in soil under the influence of Potato-Root Diffusate.

By D. W. FENWICK, M.Sc.
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When potatoes are grown in soil infested with *Heterodera rostochiensis*, the diffusates produced by the developing root stimulate the larvae to emerge from the cysts into the soil. There is reason to believe that in the presence of a susceptible host this process is very active; at the same time there is some evidence of a degree of spontaneous hatching, which occurs in the absence of a potato crop. The present paper records an attempt to obtain some quantitative measure of the extent of larval emergence in soil in the presence as well as in the absence of diffusates from a susceptible host, and also to ascertain whether or not different varieties of potatoes have significantly different effects on the degree of larval emergence. A further factor which was investigated was the effect of different soils on larval emergence.

In order to investigate these different factors as concisely as possible an experiment was set up in which potatoes were grown in 9 in. pots in a 3 : 1 mixture of loam and sand. At the time of planting the tuber, the pot was placed on top of a similar pot containing infested soil but no potato tuber; the soil surface of the latter was covered by a layer of clean gravel, the whole pot being buried up to one inch of its rim in gravel. The upper pots containing the potato tubers were heavily watered each day throughout the period of growth, so that the leachings drained into the lower pot of infested soil.

The design of the experiment was simple; three varieties of potato were planted in the upper pots, one set of pots being left without any potatoes to investigate the degree of larval emergence in the absence of potato-root diffusate. Three types of soil were used for the lower pots.

All combinations of potato variety and soil type were set up in quadruplicate replication. Potato varieties tested comprised " Majestic," " Arran Banner," " Ulster Chieftain " and a control, designated, A, B, C and D, respectively. The soils used included a stiff heavy loam occurring on the grounds of Rothamsted, a blackland peat from the Chatteris areas of Cambridgeshire as well as a light greensand from the Gamlingay area of the same county ; these were designated a, b and c, respectively. With quadruplicate replication the experiment involved 48 pairs of pots.

The three soils used for experiment were thoroughly mixed before being put up in pots and samples withdrawn for infectivity estimations. Cyst counts were based on duplicate 200 gm. samples of each soil type ; the larval counts were carried out on duplicate samples of 500 cysts, dissected and made up to 100 ccs., duplicate 1 cc. samples being examined.

Infectivity figures for the three soils were as follows :—

a. 1.54 cysts/gm., 162.6 larvae/cyst, 250 larvae/gm.
b. 1.54 cysts/gm., 93.8 larvae/cyst, 144 larvae/gm.
c. 1.39 cysts/gm., 30.6 larvae/cyst, 42.5 larvae/gm.

The experiment was set up 4th May, 1949, and ceased August 16th ; during their period of growth the potatoes suffered from the extreme heat but their development was otherwise normal. When they had died down, the experiment was dismantled, the soil in the lower pots dried and the cysts recovered. Duplicate samples of 100 cysts were withdrawn from each pot, dissected, made up to 25 ccs., and duplicate 1 cc. samples examined for eggs and larvae.

The analysis of the data thus obtained was accomplished by computing the number of eggs and larvae per cyst for each pot and expressing this as a percentage of larval content for that particular soil at the start of the experiment. This percentage was then submitted to the angular transformation, the values of ϕ thus obtained being treated as normal ordinates ; an Analysis of Variance was then performed on the data to ascertain which effects were significant.

In Table I are given the main treatment effects for the percentage of larvae left behind in the cysts at the end of the experiment. Table II is the appropriate Analysis of Variance computed on transformed data. It will be seen that both the main treatment effects, viz., soil type and potato variety have significant effects ; the interaction of soil type on potato variety is also significant. The result of the analysis can be summarised as follows. The proportion of larvae left behind in D is significantly greater than in either A, B or C and amounts to

48.2 per cent. ; fewer larvae are left in C (10.2 per cent.) than in A or B (18.9 per cent. and 17.8 per cent. respectively), which latter pair do not differ significantly from one another. The larval content of soil c appears to be significantly greater than either a or b (32.8 per cent. against 27.7 per cent. and 24.7 per cent.). The significant interaction is manifested by the fact that potato variety C seems to have caused more

TABLE I.

Percentage of larvae remaining in cysts after treatment of different soils with root diffusates from different potato varieties.

Variety / *Soil*	*A*	*B*	*C*	*D*	*Mean*
a	16.6	12.3	10.4	50.4	27.7
b	20.9	24.8	8.3	45.8	24.7
c	24.9	28.4	15.2	43.2	32.8
Mean	18.9	17.8	10.2	48.2	26.3

TABLE II.

Analysis of variance of data in Table I after conversion to ϕ.

Source	*Sums of Squares*	*D of F*	*Variance*	*V/R*
Error	712.78	36	19.8 (V_E)	
Treatments generally	4,098.88	11	372.0 (V_T)	$V_T/V_E = 18.7$ Sig.
Soils (S)	148.72	2	74.36 (V_S)	$V_S/V_E = 3.76$ Sig. $V_S/V_{SV} = 1.38$
Varieties (V)	3,627.19	3	1209.06 (V_V)	$V_V/V_E = 61.0$ Sig. $V_V/V_{SV} = 22.4$ Sig.
Interaction S×V	322.97	6	53.83 (V_{SV})	$V_{SV}/V_E = 2.72$ Sig.

larvae to hatch from soils b and c, than have A and B but it has had no similar effect on cysts in soil a. The proportion of larvae left after the action of diffusates generally is approximately 16 per cent.

A very surprising feature of these results is the large number of larvae which have hatched out from series D, that is from soils which

had not been treated with root diffusates. If the overall figure of 48.2 per cent. be taken as representing the proportion of larvae which do not hatch out in a single season, then approximately 52 per cent. of the larvae present within cysts should hatch in a similar period. If this represents the state of affairs that exists under natural conditions then it becomes possible to draw some tentative conclusions regarding the rate at which infestation of potato eelworm diminishes in the absence of a potato crop, provided it remains approximately constant over a number of seasons. Accepting the figure of 50 per cent. as an approximation to the rate of larval emergence and assuming that the larvae after emergence are not capable of surviving free in the soil for longer than a few months, then it would take two years to reduce the eelworm population to approximately 25 per cent. of its original level; after five years of resting from potato crops there would still remain over three per cent. of the original infestation, so that even then, according to Chitwood and Feldmesser (1949), it is doubtful whether it would be possible to grow more than one crop on the ground with any degree of safety. In the author's opinion, the conditions of the experiment were more favourable for larval emergence than they would be in the field, since with heavy daily watering the soil humidity in the pots would be uniformly higher. In that case, the figures quoted above for decrease in infectivity over a period of years, would be optimistic and infectivity would decline much more slowly. A further interesting feature in series D is the fact that the proportion of larvae left within the cysts appears to be remarkably constant for all three soils which suggests that in the absence of complicating factors such as ground keepers, the rate of fall of infectivity would be fairly constant for all soils.

The different degree of emergence under the action of diffusates from different varieties of potato is interesting, especially when it is realised that the diffusate inducing the greatest degree of hatching is that produced by " Ulster Chieftain," an early variety, which died down nearly a fortnight earlier than the other two varieties. That different varieties of potatoes do produce diffusates of varying power has already been demonstrated by Ellenby (1946), and the present result would indicate that his findings from experiments carried out under "*in vitro*" conditions might be of some importance on a field scale.

The interpretation of the results for the three soil types is not easy, since it is impossible to differentiate between effects due to differences in the soils and those due to differences in the cyst populations present in each soil. While no laboratory experiments have been conducted on the cyst populations present in these soils, it is not uncommon for

different batches of cysts to behave very differently from one another when treated with root diffusate ; very frequently, the differences are far greater than those experienced in this experiment. It is not inconceivable that over a period of several generations cysts may have undergone a process of adaptation to the environment peculiar to the soil type in which they have developed, and we may possibly be dealing with incipient physiological strains. It is suggested that interesting results might be obtained by inoculating different soil types with cysts from the same source as well as inoculating cysts from different sources into a single soil type. Only in this way can the relative importance of soil type and cyst type be investigated.

Summary.

An experiment was set up to investigate the effect of root diffusates from different varieties of potatoes on natural infestations of cysts in three different soils. A series was included in which no root diffusate at all was used, to obtain an estimate of the degree of spontaneous hatching which occurred. In the absence of diffusate, about 50 per cent. of larvae emerged from the cysts, as compared with 84 per cent. in the case of soils treated with diffusates. Significant differences were detected in the degree of emergence under the action of the different diffusates, that from " Ulster Chieftain " having the greatest effect. Significant differences were also detected between the different soils, larval emergence being lowest in the greensand.

Acknowledgments.

The author would take this opportunity to thank Miss Elizabeth Reid who rendered him invaluable assistance in setting up and servicing this experiment.

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Notes on the use of Iodine and Chlorphenol against certain plant nematodes.

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There is still a great need for efficient nematocides. Though the information contained in this short paper is incomplete, it is felt that publication should be made in order to inform other workers as to the stage the work has reached. It is hoped that this article may lead to further investigation on the subject and, in particular, stimulate the chemists to more work on similar types of compound, which may well bring to light a further useful range of materials. In this paper some comparisons are made between iodine solutions and " chlorphenol."

It will be conceded generally that iodine in solution (with the addition of the potassium iodide necessary for solution) is one of the substances most toxic to eelworms. Poate (1934) recommended its use at a strength of 1 in 2,000 as a means of preventing new infections of eelworm in narcissus bulbs and for the treatment of the soil from which an infested bulb had been removed ; at a strength of 1 in 80 the death of eelworms could only be described as instantaneous.

Experiments with Iodine Solutions.

The above facts concerning the use of iodine have been confirmed, using Bulb Eelworm (*Anguillulina dipsaci*) and Chrysanthemum Eelworm (*Aphelenchoides ritzema-bosi*) as test nematodes. Tests were also made with desiccated eelworms, known as eelworm " wool," from bulbs.

The results are set out in table form :—

Strength of Iodine solution	*Approximate time taken to kill all eelworms*	
1 in 2,000	2 seconds	active eelworms
1 in 4,000	10 seconds	active eelworms
1 in 16,000	3½ minutes	active eelworms
1 in 4,000	8 minutes	Eelworm " wool " from bulbs

The Treatment of Eelworm Infested Seeds.

Unfortunately, iodine is expensive and its use on any large scale is prohibited on that ground alone. It has, however, in recent experiments been found practicable to use iodine solutions for the treatment of eelworm infested seeds, since only small amounts of the solution are required. Satisfactory treatments have been worked out for eelworm infested seed of clover, teazle and onion, 10 minute soakings having no adverse effects on germination or subsequent growth. The method employed for such treatment is to place the seed in a large funnel, the spout of which is closed by a cork. A sufficient quantity of the solution is then poured on so as to cover the seed completely. After the treatment period has lapsed the cork is removed and the liquid drained away ; water is then run through the seed to remove any treatment solution which remains. The seed after draining for a few minutes is then tipped out of the funnel and well mixed with an equal volume of dry fine sand. The mixture is then spread to dry on sheets of paper and in a few hours is sufficiently dry for the sand to be separated very readily by means of a fine sieve. The seed is then re-spread and is completely dry by the next morning and ready for sowing. It is well known that the desiccated resting stage of the Stem Eelworm, known in connection with bulbs as eelworm "wool," is outstandingly resistant to heat and toxic chemicals ; individual eelworms vary in their degree of susceptibility. Examination of treated infested seed showed that a complete kill had been obtained. Germination tests and growing of treated and untreated seed showed that germination and subsequent growth were unimpaired. Treatment with iodine solution was found to be equally satisfactory in the case of teazle and onion seed.

Experiments with Chlorphenol.

In view of the expense of iodine, a search was made for some alternative substance ; this was found in 1940 in the form of technical material containing chlorphenol ; until now, no publication on the subject has been made. The information set out in this paper refers to chlorphenol material generously supplied by Messrs. British Alkaloids Ltd. (Manufacturing Chemists). Thanks are due to them for the supply of the material and also for furnishing information as to its composition, as follows :—

The material contains both orthochlorphenol and 2-4 dichlorphenol in about the proportions of 70 and 30 per cent. respectively ; it also

contains about 6 per cent. of water and $\frac{1}{4}$ per cent. of free hydrochloric acid. The mixture is soluble in water to the extent of about $\frac{1}{2}$ per cent. In this article the term " chlorphenol " will refer to the above-mentioned material.

Thanks are also due to British Alkaloids Ltd. for preparing a sample of chlorphenol material with a higher chlorine content (nearly 40 per cent.); this will be referred to as " dichlorphenol "; the solubility in water is also about $\frac{1}{2}$ per cent. The boiling points of these two materials are between 200–212°C. and the differences from one to the other are slight.

TOXICITY OF " CHLORPHENOL " AND " DICHLORPHENOL " TO EELWORMS.

Tests were made on active eelworms of two species, *Anguillulina dipsaci* from bulbs and *Aphelenchoides ritzema-bosi* from chrysanthemums. No appreciable difference was found between the two species. The results of these tests are set out in table form below :—

Strength of solution of chlorphenol material	*Time taken to kill the most resistant individuals in the sample of eelworms*	
$\frac{1}{2}$ per cent. " chlorphenol "	2 minutes	Active eelworms of both species
$\frac{1}{4}$ per cent. " chlorphenol "	5 minutes	
$\frac{1}{8}$ per cent. " chlorphenol "	7 minutes	
$\frac{1}{2}$ per cent. " chlorphenol "	15 minutes	Desiccated " wool " stage of *A. dipsaci*
$\frac{1}{4}$ per cent. " chlorphenol "	40 minutes	
$\frac{1}{2}$ per cent. " chlorphenol "	1 hour	Desiccated stage of *A. ritzema-bosi* in dried chrysanthemum leaves. (This includes an unknown period of time for the penetration of the solution into the leaves; but as the leaves became limp in a few minutes penetration probably did not take much longer.)

In all the above experiments the eelworms were kept for up to 5 days in shallow clean water before a final assessment of their condition was made.

When " dichlorphenol " was used at the same strengths, the times for killing were reduced by approximately one third.

The action of " chlorphenol " solutions on eelworms is of interest when compared with the effects of iodine solutions. In the case of iodine, the eelworm first becomes more active and then rapidly, but evenly, loses activity until it lies straight and dead. When " chlorphenol " is used the eelworm is not immediately stimulated but soon assumes, suddenly, a typical " kinked " attitude ; it then lies quiet again for short periods, interspersed with sudden convulsions of a typical kind ; these give the impression of a spring under tension suddenly released. After each convulsion the eelworm lies still in the " kinked " attitude and finally lies straight when dead. The convulsions are even more striking in the case of " dichlorphenol."

Experiments on the Treatment of Seeds with " Chlorphenol."

Having obtained information on the killing of stem and bulb eelworms in the desiccated stage, a series of experiments in the treatment of clover and teazle seed was carried out. The method of treatment of the seed was the same as has been described for iodine solutions.

It was found that clover seed would readily stand immersion in a $\frac{1}{2}$ per cent. " chlorphenol " solution for 20 minutes without any reduction in percentage germination, though germination was delayed about 3 days ; the same result was obtained with teazle seed. With both these seeds a test was made, by treating infested samples, to determine that all eelworms were killed. In addition to the ordinary incubated germination tests, further samples of seed were treated and grown in boxes until the 4-leaf was reached. Growth was normal and a total plant equal to the controls was obtained. The work was then extended by treating enough infested clover seed to sow $\frac{1}{4}$ acre of land which had not grown clover before. Despite the fact that the soil was dry and in poor seed-bed condition, a very good clean plant was obtained. Random samplings of the plant were examined by means of Baermann funnels as a check on field observations, but no eelworms could be found.

Several pounds of heavily infested teazle seed, from the same sample which had grown a very poor crop of teazles, were also treated with a $\frac{1}{2}$ per cent. " chlorphenol " solution and grown under field conditions in the Langport area of Somerset where this crop is still grown commercially. The serious trouble known as " Cabbagy Plant " has now been proved to be due to the attacks of the Stem and Bulb eelworm

(*Anguillulina dipsaci*) and it is now known that the eelworm is seed-borne. Teazles have been grown for many years on the farm where the treated seed was sown and it is known that certain weed hosts are involved. The treated seed-bed was, however, for all practical purposes free of eelworm infestation ; though a few isolated infested plants were found near the headland. The previous year this same seed gave a seed-bed which was infested to an extent of about 70 per cent., large patches of the seed-bed failing completely to provide any normal plants for transplanting. The treatment of the seed did not affect germination or vigour of subsequent growth.

THE USE OF " CHLORPHENOL " IN THE HOT-WATER BATH FOR THE CONTROL OF EELWORM IN NARCISSUS BULBS.

The " wool " stage of the eelworm is of the utmost importance when infested bulbs are treated in the hot-water bath. Bulb growers sometimes state that they do not, in practice, always get complete control of eelworm by such treatments even from a full 3-hour treatment ; they sometimes attribute this to some of the eggs escaping treatment, which is unlikely ; for previous work by the writer has shown that eggs are the most easily killed of all the stages of the eelworm. Alternatively, the assertion is made that some of the eelworms in the " wool " stage survive the treatment. Since the " wool " stage is killed in 78 minutes at a temperature of 110°F. and supposing very large bulbs which take 1½ hours to heat through to the centre, where " wool " is not commonly found, then there will still be a margin of 12 minutes as an insurance for complete control. This margin may seem small, but " wool " is largely on the outside of the bulbs and very quickly receives heat and, in particular, the heat from water of about 115°F., this being the common initial temperature for the water when the bulbs are first loaded into the bath.

It was thought very likely that air pockets might form around "wool." Air pockets might also form, more readily, as the result of semi-dried bulb tissue in severely affected bulbs, which are often also attacked by fungi. It has frequently been observed that bubbles of air rise throughout the whole of a 3-hour treatment. It was found that the addition of a wetting compound as used in an insecticide spray, resulted in practically all air bubbles ceasing to appear after 5-10 minutes. Wetters had no ill effects on the growth of the bulbs.

The experiments with " chlorphenol " on eelworm " wool " clearly opened up the possibility of adding the material to the water in the bath so as to be sure of a complete kill.

A strength of ¼ per cent. " chlorphenol " was considered ample, since it would kill all " wool " in the cold, in 40 minutes and so, leaving out of consideration all questions of heat, give a margin of 140 minutes during a 3-hour treatment. Small scale treatments with the addition of the " chlorphenol " and wetting agent were carried out in the laboratory on bulbs heavily infested with eelworm in the " wool " stage. Complete control was obtained. Several 10 cwt. lots of heavily infested bulbs were treated on a commercial holding in Cornwall. When grown on, the bulbs behaved normally and eelworm control, as observed during the remainder of the growing season, was apparently complete.

During these treatments a ¼ per cent. strength solution of " chlorphenol " was also used to wash over all staging, walls, floors, etc., of the shed where the hot-water treatments were carried out ; formalin and several other disinfectants are of doubtful value for this very important part of the hot-water treatment process ; it is considered that a solution having a known positive action on eelworms is desirable. Growers are not yet fully aware of the many ways in which eelworm is capable of spreading from one part of a holding to another, and too often doubt is cast on hot-water treatment when the causes of spread or re-infestation should be sought elsewhere.

Experiments on the Phytocidal Action of " Chlorphenol."

Since bulbs are not in a state of active growth during the hot-water treatment season they cannot be regarded as a good test for phytocidal action ; it was considered that " chlorphenol " might prove of value for the destruction of weed hosts, together with the eelworms infesting them, should the material prove to be strongly phytocidal. If " chlorphenol " was not phytocidal then the scope of the material might be extended in other directions.

Since no ill effects arose from the treatment of clover and teazle seed, the question as to the germination of seeds in land treated with chlorphenol was tested on three small plots. One plot was untreated, but the other two were each watered with ½ per cent. and ¼ per cent. solutions of chlorphenol respectively, at the rate of ½ gallon per square yard. After watering, the surface of the soil was undisturbed except for a light raking just before the seeds were sown. Seeds of mangold and kale were sown in all three plots, 1, 2 and 3 weeks after treatment. No ill effects were noted on the germination of the seed or upon subsequent growth. Another plot was soaked with ½ gallon of ½ per cent. chlorphenol per square yard and kale and mangold seed were

then sown 24 hours later. The seeds again germinated and grew without any ill effects.

To test whether the actual spraying of weeds would result in damage, ½ per cent. chlorphenol was sprayed with a fine nozzle on Ground Elder, Dandelion and Nettles. No serious phytocidal action occurred, but there was some marginal scorching.

"CHLORPHENOL" AND POTATO ROOT EELWORM. (*Heterodera rostochiensis.*)

The toxicity of "chlorphenol" to eelworms clearly opened up the possibility of extending its use in connection with such problems as potato root eelworm.

It was found that potato eelworm cysts soaked in a ½ per cent. solution of "chlorphenol" for 30 minutes could not be induced to hatch by means of potato root diffusate, whereas untreated cysts hatched well. Soil containing cysts was similarly treated; some of the cysts were washed 24 hours after treatment and others after 72 hours; some were not washed. A series of daily hatching tests with root diffusate gave continuous hatching from untreated cysts over a period of 14 days; no eelworms hatched from any of the cysts from the treated soil.

This experiment suggested that cysts were either killed or hatching was delayed. Pot experiments were then carried out using eelworm infested and clean soil, each series being divided into treated and untreated sets. For the treated pots sufficient ½ per cent. "chlorphenol" solution was poured into each to soak the contained soil. Treatment of the soil was made in early May and all the pots were planted with sprouted potato sets early in June. Later it was observed that the treated pots led to severe yellowing of the potato plants and it was found that the roots next to the surface of the pot had been killed, the pots evidently having absorbed the "chlorphenol" and caused a local concentration. Other roots were unharmed and it is probable that no root injury would have taken place in open ground. Eelworm was not controlled, being approximately equal in treated and untreated plants. Treated plants, however, made more root and, had not this root been injured, might have given a greater crop.

Later on, cysts were soaked in ½ per cent. "chlorphenol" for up to 1 hour and the "kill" estimated by the Bracey method. In these experiments no apparent "kill" took place. Longer soakings, up to 21 days, were then tried with the addition of a wetter at ½ per cent. strength. Examinations by the Bracey technique gave a figure of 85

per cent. " killed ; " when " dichlorphenol " at the same strength was used the " kill " was increased to 92 per cent. The control figure for untreated cysts was 4.5 per cent.

It was felt that these results were sufficiently encouraging to be applied on a small field experiment. It was thought that some control might be obtained if the " chlorphenol " could be introduced into the soil in such a way that it gradually went into solution over the growing period, preferably the early part.

A dust was therefore used which consisted of an inert carrier with which was incorporated 15 per cent. by weight of " chlorphenol." This was applied at two different dressings, the best results being obtained with the smaller rate of 1½ lbs. of the dust to 9 feet of potato row in a strip 1 foot wide ; the dust was then well mixed with the top 6 inches of soil. " Chlorphenol " is only slowly soluble in water and it was hoped a small concentration of the material in solution would continue to be present for some time after planting and so control larvae as they hatched. Unfortunately the season was exceptionally dry and the soil was light and very hot. The plants in the treated soil all grew and were of a good green colour. On untreated soil typical severely stunted " eelworm plants " were produced. The yield from the treated plants was about 1½ times that of the controls. It is of interest that good growth and no root injury took place where the application of the " chlorphenol " was of the order of 1,000 lbs. per acre ; also that no tainting of the tubers occurred.

Cyst and egg counts before and after the experiment, on control and treated plots, showed an increase and in this way the results are somewhat similar to those obtained in various experiments with D.D. Nevertheless, it is thought that the incorporation of a nematocide in a dust which can be thoroughly mixed with the soil may be a possible means of combating this pest and the experiment is therefore recorded.

Summary.

1. " Chlorphenol " and " dichlorphenol " are shown to be highly toxic to eelworms, both in the active and quiescent stages. Comparisons are made between these chemicals and iodine solutions in this respect.
2. Treatments of clover and teazle seed with " chlorphenol " and iodine solutions are described and it is shown that infesting eelworms may be controlled without affecting germination or subsequent growth.

3. The use of " chlorphenol," with the addition of a wetting agent is recommended for the hot-water treatment of narcissus bulbs, as an additional insurance for the control of the " wool " stage of bulb eelworm.
4. The phytocidal action of "chlorphenol" is discussed and it is shown that such action is not marked.
5. Some preliminary experiments on the use of " chlorphenol " against potato root eelworm are described.

ACKNOWLEDGMENTS.

The writer wishes to make full acknowledgment of the assistance given by Mr. L. E. W. Stone, who was largely responsible for carrying out the work with potato root eelworm. Miss E. Britton and Mr. J. Southey assisted with some of the work on stem eelworm. Mr. E. D. Wiggins gave similar assistance with potato eelworm work when he was in the writer's department at Seale Hayne Agricultural College. Thanks are due to all these assistants for their ready help and interest. Dr. H. G. H. Kearns of Long Ashton Research Station very kindly made up the chlorphenol dust.

REFERENCE.

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Further Observations on the Seasonal Variation in Worm Egg Output in Scottish Hill Sheep

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The prevalence of helminths in sheep and the need for more exact methods for their control have stimulated researches in many countries into the problems of helminthiasis under grazing conditions. Not only have the factors concerned in serious outbreaks of helminthic disease been studied, but the more insidious and more prevalent subclinical aspects are receiving increased attention. Indeed, it is now generally accepted that the losses, particularly in meat and wool, resulting from subclinical helminthiasis present a greater economic problem than obvious parasitic infestations. In this connection the use of prophylactic anthelmintic treatment, or "strategic" dosing (as it is now often called), and control of worms by grazing and pasture management are of vital importance. Unfortunately, extensive areas of Scottish and English hill pastures, carrying a large population of sheep, do not lend themselves to the generally accepted methods of control of parasitic diseases by grazing management, and therefore control by dosing with anthelmintics would appear at present to be the only method which can be applied to flocks in these areas. This, however, raises problems which are both practical and economic ; and it is unlikely that many hill farmers would embark on a scheme of control involving frequent dosings of whole flocks. Two or three dosings a year might be undertaken but these should, where possible, be arranged to coincide not only with the normal gathering of the flocks but essentially with the time when the anthelmintic is likely to have the maximum effect. It follows, therefore, that a quantitative and qualitative study of the seasonal variations in the worm burden of sheep is essential before a scheme of strategic dosing, at once practical and effective, can be recommended.

Morgan and Sloan (1947) studied this problem in so far as it related to sheep under Scottish hill conditions, where the rather more standardized methods of management are less likely to introduce factors which might interfere with normal seasonal differences in the worm burden of the host. Their results show quite clearly that the pattern of the worm burden, in so far as it is revealed by the eggs passed in the faeces, is fairly constant. They state that :—" There is a very marked increase in output of helminth eggs during the spring." " Lowest levels occur in the winter months." " The spring increase is clearly shown in all age groups." " Heaviest infestations are found in the hoggs, followed by gimmers."

A similar marked increase in egg production in the spring, but under entirely different conditions, had been observed by Taylor (1935) in England, Hawkins *et al.* (1944 and 1947) and Segetti and Marsh (1945) in the United States and, since the work by Morgan and Sloan, by Cushnie and White in Scotland (1948) and Naerland (1949) in Norway.

The studies made by Morgan and Sloan were restricted to two typical hill farms in the Ettrick district of the Scottish Borders and extended over a period of two years. The results obtained were considered of sufficient importance to warrant an extension of the observations in order to discover whether or not this phenomenon was confined to the farms where the studies had been made. It was decided, therefore, to continue the investigation on the same hefts* in Ettrick, and simultaneously to extend the work to hill farms situated in other districts of Scotland. Farms were selected in Wigtownshire, Dumfriesshire, Lanarkshire, Stirlingshire, Argyllshire, Midlothian, East Lothian and Berwickshire, where periodic worm egg counts were made on representative hefts.

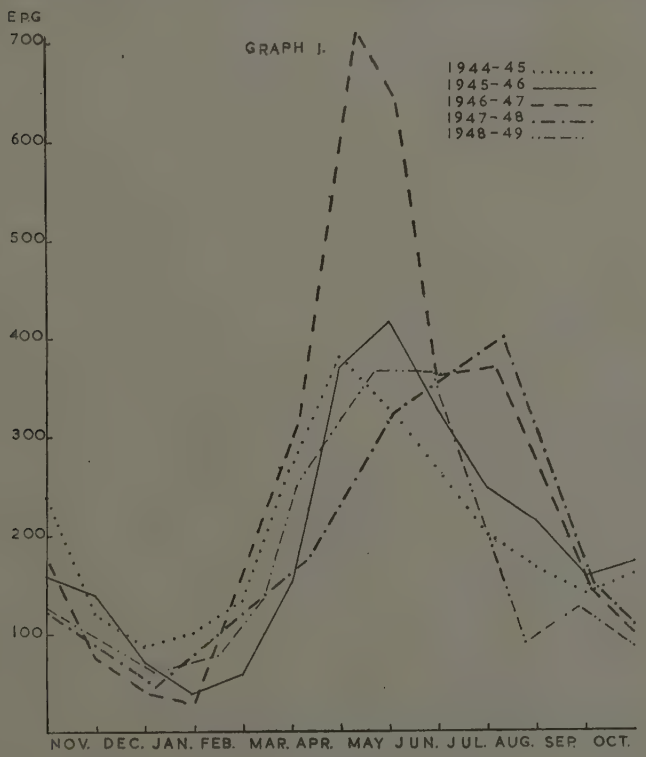
Material and Methods.

In most cases the sheep were numbered with earrings so that they could be identified throughout the period of investigation. Samples of faeces were taken from the rectum of each sheep and were then transported to the laboratory in numbered tin boxes for examination. The egg counting technique used was that devised by Gordon and Whitlock (1939) with certain modifications. Two grammes of faeces were weighed and rubbed down on a sieve resting in a broad evaporating basin containing 30 cc. of saturated salt solution. A further 30 cc. of the salt solution was then used to give a final washing of the remains of

* A heft is a part of a hirsel or flock, consisting usually of a few score of sheep, which has the exclusive grazing of a well-defined section of a hill and which does not stray from that part of the hill.

the faecal sample left on the sieve. Counts were made on four cells of the McMaster slide and the eggs were grouped under the categories, "Strongyloides eggs," "Nematodirus eggs" and "Strongyle eggs;" the totals multiplied by 50 gave the number of eggs per gram. of faeces.

It was not always possible to obtain faecal samples from every sheep,



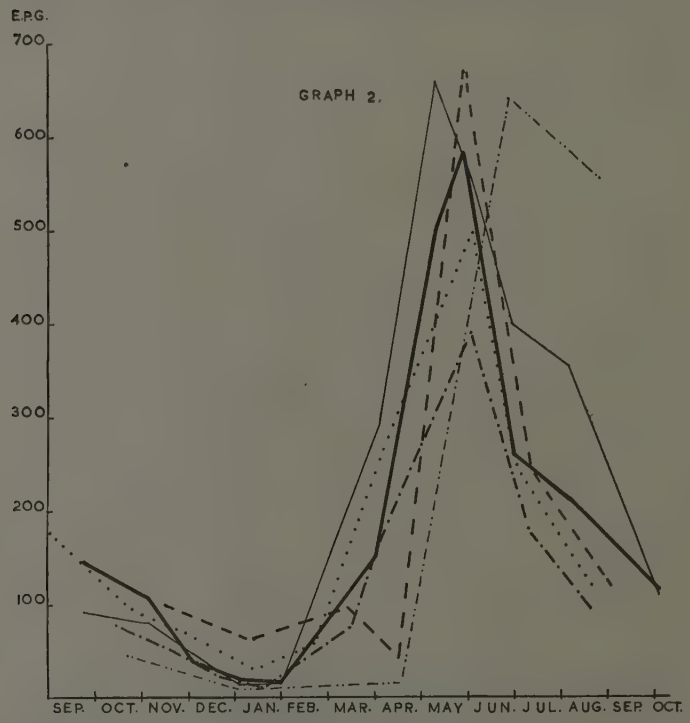
Graph 1. Annual variations in the average worm egg output of sheep of all ages in the two Ettrick hefts.

since it sometimes happened that one or two sheep were not brought in from the hill owing to difficulties in gathering, particularly in bad weather; occasionally also no faeces were present in the rectum.

It is unfortunate that after the first two weeks of April no sampling can be done readily on breeding ewes until about the last week of May.

This is because lambing commences about the middle of April, and during the greater part of May the gathering of very young lambs involves practical difficulties.

While in the main these studies were made on hefts composed of the Scottish Blackface breed of sheep some sampling was done on the



Graph 2. Worm egg averages for ewes on six hefts during 1946–47; the two solid lines represent the two Ettrick hefts.

Cheviot breed; results obtained from the latter are included in this paper. In all 1,470 sheep were studied periodically during this investigation; no anthelmintic treatment was carried out on any of these sheep.

The results are presented under three main designations, viz. "hoggs," "gimmers" and "ewes," terms related to age groups in the female sheep. For the purpose of this paper the term "hoggs"

denotes sheep ranging in age from 6 months to 18 months, " gimmers " from 18 months to 2 years and 6 months, and " ewes " includes all the older age groups. No extensive observations have so far been made on lambs, a term representing the period from birth in April until about September, when the more suitable females are selected to be included in the hill flocks and are called " ewe hoggs." Usually ewes are sold off the hill farms when they are five or six years old, and are referred to as " draft " or " cast " ewes.

Results.

Worm egg counts on the two hefts in Ettrick.

The investigation on the two hefts of sheep in Ettrick which were sampled by Morgan and Sloan during 1944–1946 has been continued in the same manner, except that samples were collected less regularly.

Graph 1 gives the average egg counts for all ages on both hefts and includes the data from November, 1944, to October, 1946, given by Morgan and Sloan (in Graph B of their paper), as well as the results for each year from 1946 to 1949. It will be seen that in the spring of 1947 the worm egg counts on the two hefts averaged over 250 eggs per gram. higher than they did in the previous or the following years. This may have been due to an exceptionally severe winter followed by a cold and wet spring in 1947. The only other differences of note were the delay in the fall of the egg output in the summer of 1948, which was wet and sunless following a dry May, and the rapid fall in the summer of 1949, which was unusually dry and sunny. These differences were noted in all the age groups.

It should be noted that the graphs for the two years 1944–45 and 1945–46 are not strictly comparable with those for the years 1946 to 1949, since Strongyloides and Nematodirus eggs are included in the totals for the latter years. This difference, however, does not appreciably alter the general picture of the seasonal variations since it amounted in all to an average of only three eggs per gram. of faeces in January, 1947, and eight eggs in January of the two following years, 1948 and 1949 ; In June, during the three years, 1947, 1948 and 1949, the average was increased only by 20 to 25 eggs per gram., i.e., about 5 per cent. of the total count.

Worm egg counts on hefts of Blackface sheep from other districts of Scotland during 1946–48.

In addition to the two hefts in Ettrick, it was planned to sample eight other hefts of Blackface sheep at monthly or quarterly intervals during 1946–47. Unfortunately, the sampling was seriously interrupted

in 1947 by the unusually heavy snowfalls which made it impracticable to reach some farms, and on others made it impossible to gather the sheep. As a result of this interruption it is probable that the period of highest egg production was missed on those farms ; nevertheless the results show an overall picture, at least for the ewes and gimmers, which is similar to that obtained on the Ettrick hefts.

In 1946–47 the data were based on some 620 ewes, 200 gimmers and 170 hoggs and in the following year on 290 ewes, 100 gimmers and 180 hoggs.

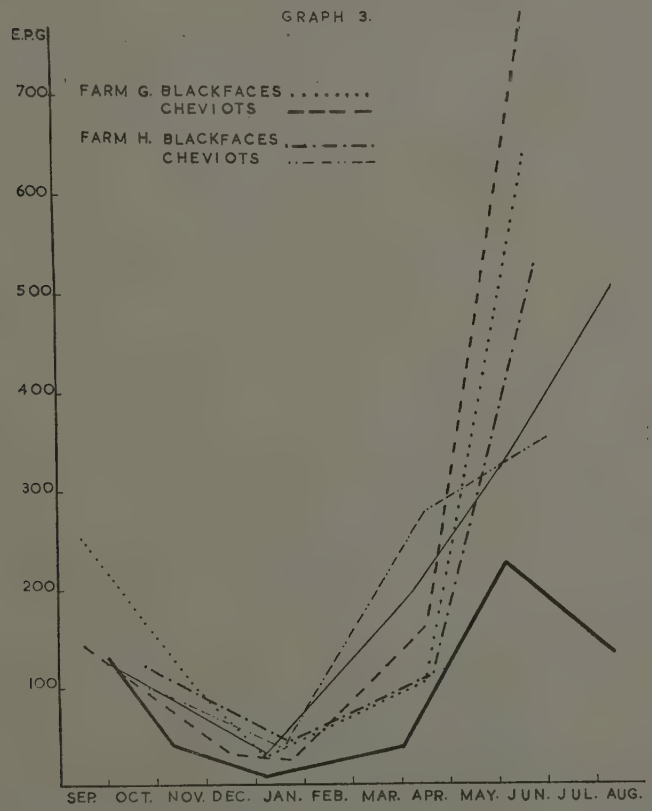
Ewes 1946–47. The results for the ewes from six of the ten farms are shown in Graph 2. Without exception each shows that the miniumum egg production—varying from 17 to 70 eggs per gram. —occurred in early winter after a steady fall during Autumn. Again, without exception, the highest egg counts—varying from 241 to 879 eggs per gram. of faeces—were obtained in late spring. After reaching its peak the egg output fell very rapidly and by about August or September it reached a level below 150 eggs per gram. On four of the ten farms, owing to the severe weather, there were long gaps between sampling in the first half of the year, and the results are therefore not included in Graph 2 ; but the data show that there was an increase in worm egg output after the low level of early winter.

Ewes 1947–48. Observations on six hefts, including two of Cheviot sheep, were made in 1947–48. The results for the ewes show a low level of worm egg output in January and a rise by late spring, which in some hefts reached a fairly high level. This is illustrated in Graph 3.

Gimmers. The separation of the data for gimmers from those of the older sheep is due partly to the findings of Morgan and Sloan (1947) who state that the general level of the egg output in this age group is higher than that of the ewes ; and partly to the fact that in hill sheep this is generally the youngest age group to produce lambs, and accordingly gimmers are subjected to a physiological stress which may influence their resistance to helminths.

Gimmers 1946–47. Graph 4 shows the results obtained for gimmers from six farms in 1946–1947 ; these sheep formed part of the same hefts as the ewes which were studied in that year (see Graph 2). In general the seasonal variation is similar to that obtained for the older age groups. Here again the egg output was at its lowest about January when it varied from almost none to 96 eggs per gram. of faeces, but by May, June and July higher averages were obtained than in the ewes ; on some farms these reached nearly 1,600 eggs per gram of faeces.

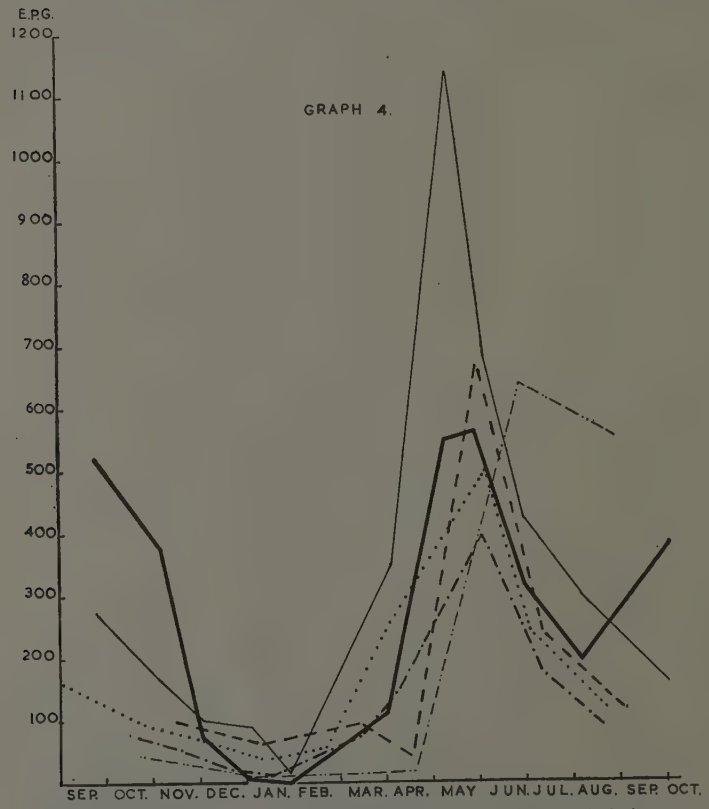
Gimmers 1947–48. The data obtained for the gimmers in the following year, 1947–48, for the two Ettrick hefts and for the two farms where Blackface and Cheviot breeds were compared (Graph 5) reveal the fact that not only during the spring rise but also in winter the



Graph 3. Worm egg averages for ewes on six hefts during 1947–48; the two solid lines represent the two Ettrick hefts.

worm egg averages were higher than in the ewes. It must be noted, however, that the number of gimmers sampled in each farm represented only about a third or a quarter of the total number of ewes under investigation.

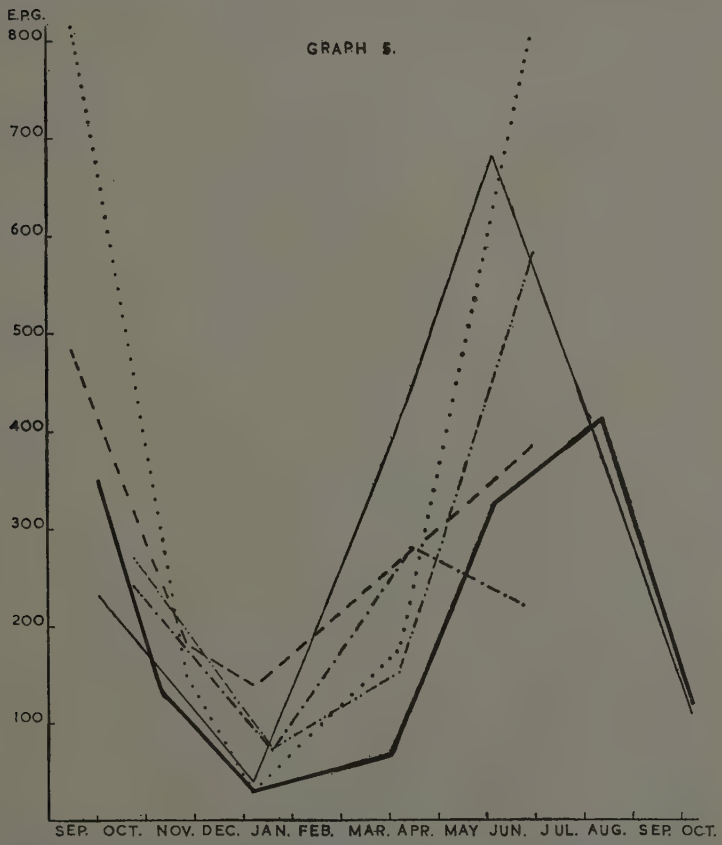
Ewe Hoggs. The management of ewe hoggs varies on farms in different districts, and very often on different farms in the same district, but flockmasters appreciate the importance of rearing their



Graph 4. Worm egg averages for gimmers in 1946–47 on the same six hefts as are illustrated in Graph 2.

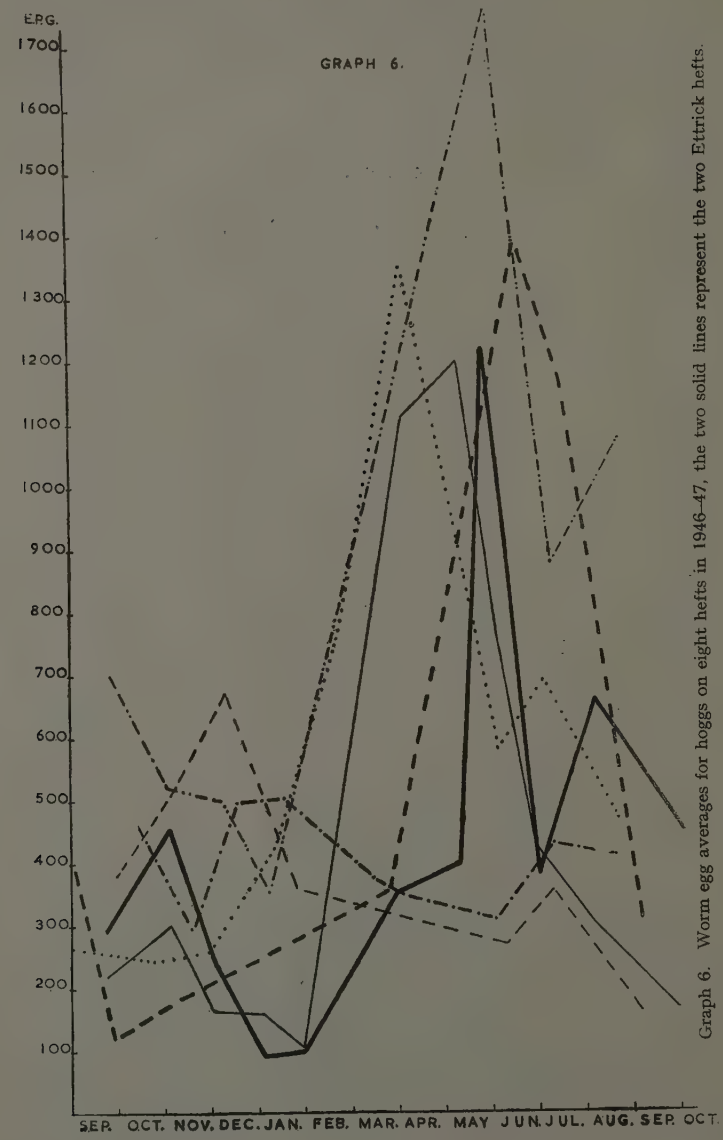
young stock well. Some hoggs are wintered on the hill, others on the home low ground and others are sent away for wintering to a lowland farm. In this paper, however, only hoggs wintered on the hill under the same conditions as the rest of the flock are considered.

Ewe Hoggs 1946–47. Graph 6 represents the data for Blackface ewe hoggs on eight farms during 1946–47. The results in this age group were less constant than in older sheep. On five of the farms there was



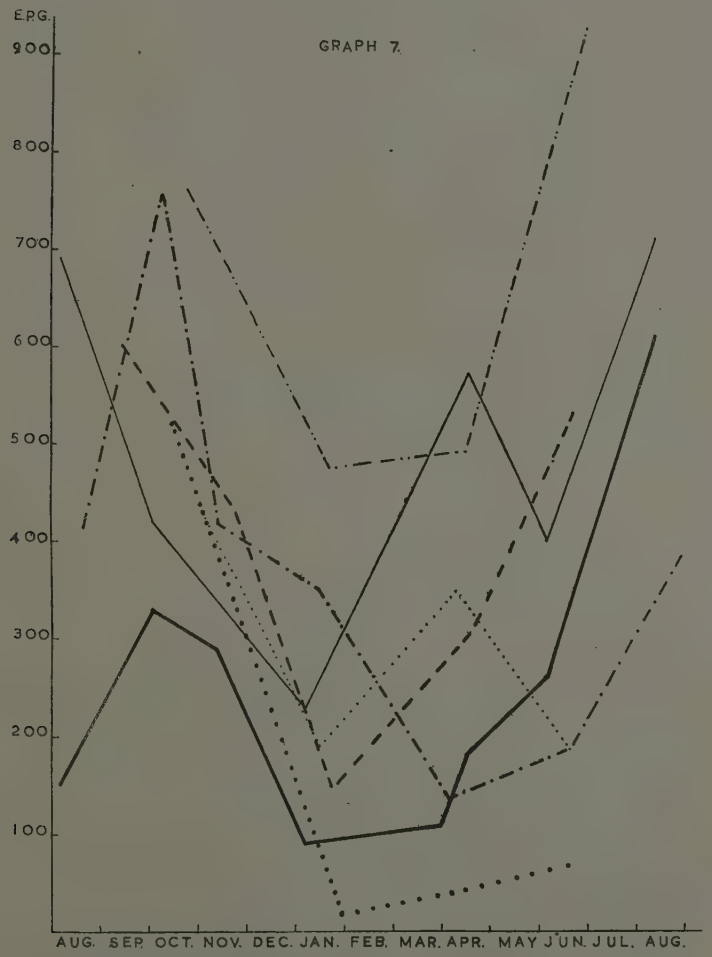
Graph 5. Worm egg averages for gimmers in 1947–48 on the same six hefts as are illustrated in Graph 3 ; the Blackface and Cheviot hefts are represented in the same way as in Graph 3.

a very marked spring rise in worm egg output ; on three of the farms there was no evidence of a spring increase, but on two of these the periods between samplings were too long to enable definite conclusions



Graph 6. Worm egg averages for hoggs on eight hefts in 1946-47, the two solid lines represent the two Ettrick hefts.

to be drawn. Graph 6 also shows that the level of worm egg output was higher in winter and, where it occurred, tended to a higher peak in the spring than in older sheep.



Graph 7. Worm egg averages for hoggs on seven farms in 1947–48 ; the two solid lines represent the two Ettrick hefts and the dash dot line (-.-.-.-) the same farm that this line represented in the previous year.

Ewe Hoggs 1947–48. In view of the fact that an exact knowledge of the seasonal egg output in this age group is of paramount importance in any future scheme of helminth control and that the seasonal pattern of egg production in the hoggs was not too clearly defined, it was decided to continue observations on this age group for another year. The results for 1947–48 cover seven farms and are shown in Graph 7 ; on the two farms where both Blackfaces and Cheviots were sampled they have been averaged together. The worm egg output was even more variable than in 1946–47 and no definite conclusion could therefore be drawn, although it must be noted that the winter output of eggs seems again to be relatively greater than in older sheep. It is still considered, however, that the underlying pattern, although erratic, is the same as that shown in older sheep.

On some of the farms a high level of egg output was maintained well into the summer of 1948 ; this may have been influenced by the wet and sunless June of that year.

In 1946–47 and in 1947–48 the ewe hoggs of the farm on the Pentlands, where about fifty were sampled each year, gave no increase in the average worm egg output in the spring ; (in Graphs 6 and 7 this farm is indicated by — . — . — . — . —).

Comparison between Worm egg output of Blackfaces and Cheviots during 1947–48.

Since all the earlier work on the seasonal pattern of egg output had been done on Blackfaces, it was decided to extend the observations to find out whether or not the same pattern occurs in Cheviots on Scottish hills. Two farms were studied where both breeds were kept under comparable conditions.

Graph 3 (which includes two other Blackface hefts) gives the average worm egg production of ewes belonging to both breeds on these farms ; Graph 5 shows the corresponding data for the gimmers. Again the lowest worm egg output occurred early in the year, with a marked rise in the spring. No marked difference was found in the egg output between the two breeds.

The comparison between the ewe hoggs of two breeds on these two farms is given in Table I ; unfortunately, there were not many Blackface hoggs included in the second farm.

Distribution of degrees of infestation as shown by worm egg counts.

That the seasonal pattern shown in Graphs 1 to 7 is not due to a few individual sheep with unusually high infestations is shown in Tables II, III and IV where the percentages of sheep falling into a series of four degrees of worm egg output are tabulated.

It is exceedingly difficult to lay down standards of egg output indicating intensities of infestation ; but from experience of helminths in Scottish hill sheep the following divisions are considered reasonable:—

0–100 worm eggs per gram. of faeces indicates a very light infestation.

150–450 worm eggs per gram. of faeces indicates a light infestation.

500–950 worm eggs per gram. of faeces indicates a medium infestation.

1,000 and over worm eggs per gram. of faeces indicates a heavy infestation.

Table I.

Worm egg counts of Blackface and Cheviot ewe hoggs.

	No. of sheep	Mid Sept.	Mid Oct.	Mid Nov.	Mid Jan.	Mid Apr.	Mid-end June
Blackfaces on farm I ..	18	611	—	480	121	250	737
Cheviots on farm I ..	18	591	—	394	177	360	806
Blackfaces on farm II ..	7	—	785	—	450	480	637
Cheviots on farm II ..	14	—	745	—	485	491	1,040

In these counts Strongyloides, Nematodirus and " Other Strongyles " have been included ; but the eggs from the two former seldom have a marked influence on the worm egg counts of adult hill sheep.

Tables II to IV show the percentages for each degree of worm egg output for the two years 1946–47 and 1947–48 ; the latter includes Cheviot as well as Blackface sheep.

In 1947, 82 per cent. of the ewes on farms where the sheep were sampled regularly showed a spring rise of 100 eggs or more per gram. of faeces, and in 1948, 70 per cent. showed a similar rise. Comparable percentages for gimmers and ewe hoggs were 80 and 74 in 1947, and 75 and 54 in 1948.

The standards of egg output of hill sheep as represented in these tables are low as compared with the output of low ground sheep.

TABLE II.

The number of ewes examined and the percentage with very light, light, medium, and high worm egg counts.

Year	No. of	Eggs per gram. of faeces	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	Early April	June	July	Aug.	Early Sept.
1946–47	Farms	—	4	6	8	5	9	3	4	5	9	9	7	3
	Ewes	—	206	358	459	322	540	260	234	289	477	463	327	142
	—	0–100	69.9	79.1	87.1	91.6	95.7	92.5	76.9	66.4	35.2	55.3	55.6	77.4
	—	150–450	23.8	19.3	10.7	8.1	3.9	6.9	18.4	21.8	32.1	30.9	29.7	19
	—	500–950	3.4	1.4	1.1	0.3	0	0.6	4.3	6.9	16.1	9.1	9.5	2.8
	—	1,000 and over	2.8	0.3	1.1	0	0.4	0	0.4	1.4	16.5	4.8	5.1	0.7
1947–48	Farms	—	1	3	2	—	4	—	—	4	4	—	2	—
	Ewes	—	78	214	119	—	274	—	—	251	244	—	102	—
	—	0–100	51.3	68.2	88.2	—	93.8	—	—	66.5	40.9	—	53.9	—
	—	150–450	39.7	27.2	9.2	—	5.8	—	—	26.3	35.2	—	35.3	—
	—	500–950	6.4	4.2	2.5	—	0.4	—	—	5.2	14.3	—	6.9	—
	—	1,000 and over	2.6	0	0	—	0	—	—	2.0	9.4	—	3.8	—

TABLE III.

The number of gimmers examined and the percentage with very light, light, medium, and high worm egg counts.

Year	No. of	Eggs per gram. of faeces	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	Early April	June	July	Aug.	Early Sept.
1946–47	Farms	—	4	6	8	5	9	3	4	5	9	9	7	3
	Gimmers	—	75	111	148	99	165	42	97	103	163	152	111	41
	—	0–100	40.	66.7	81.1	84.8	89.7	92.9	78.4	61.2	33.1	46.	58.7	68.3
	—	150–450	36.	28.8	12.2	13.1	7.9	7.1	20.6	30.1	34.4	31.6	26.1	24.4
	—	500–950	10.7	4.5	4.1	1.	2.4	0	1.	7.8	17.2	9.9	9.9	2.4
	—	1,000 and over	13.3	0	2.6	1.	0	0	0	0.9	15.3	12.5	7.2	4.9
1947–48	Farms	—	1	3	2	—	4	—	—	4	4	—	—	2
	Gimmers	—	25	50	39	—	71	—	—	68	63	—	—	23
	—	0–100	16.	34.	61.5	—	77.5	—	—	58.8	36.5	—	—	17.4
	—	150–450	40.	40.	30.8	—	21.1	—	—	26.5	30.2	—	—	56.5
	—	500–950	28.	24.	7.7	—	1.4	—	—	10.3	19.	—	—	21.7
	—	1,000 and over	16.	2.	0	—	0	—	—	4.4	14.3	—	—	4.4

TABLE IV.

The number of hoggs examined and the percentage with very light, light, medium, and high worm egg counts.

Year	No. of	Eggs per gram. of faeces	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	Early April	May	June	July	Aug.
1946–47	Farms	—	—	—	2	2	4	3	4	3	1	4	2	4	4	3
	Hoggs	—	—	—	32	64	89	70	80	31	43	71	23	77	74	70
	—	0–100	—	—	18.8	17.2	24.8	12.9	23.7	45.2	20.9	18.3	4.3	22.1	22.9	34.3
	—	150–450	—	—	62.5	48.4	53.9	54.3	47.5	38.7	51.2	43.7	47.8	35.1	37.8	31.4
	—	500–950	—	—	18.8	31.3	19.1	24.3	21.2	9.7	23.3	23.9	13.2	25.9	20.3	14.3
	—	1,000 and over	—	—	0	3.1	2.2	8.7	7.7	6.4	4.6	14.1	34.9	16.9	18.9	19.9
1947–48	Farms	—	1	1	1	6	3	—	7	—	—	7	—	7	—	3
	Hoggs	—	65	55	35	153	101	—	166	—	—	171	—	165	—	61
	—	0–100	46.2	36.4	0	16.9	11.9	—	51.2	—	—	48.5	—	48.5	—	36.1
	—	150–450	43.1	40.	54.3	41.2	56.4	—	35.5	—	—	35.7	—	31.5	—	40.9
	—	500–950	9.2	12.7	34.3	25.5	25.7	—	10.8	—	—	12.3	—	12.1	—	11.5
	—	1,000 and over	1.5	10.9	11.4	16.3	5.9	—	2.4	—	—	3.5	—	7.9	—	11.4

but factors such as the size of hill sheep, their low level of nutrition, and the low incidence of the prolific egg layer *Haemonchus contortus* may have a bearing on the significance of the egg count in relation to clinical and subclinical helminthiasis.

Meteorological Data.

Weather has an undoubted influence on the well-being of stock on the hills, and, as already suggested, may have a bearing on the height and duration of the spring peak of worm egg output.

The following meteorological data were obtained from the Observatory in Eskdalemuir, which is situated only a few miles from the farms sampled in Ettrick.

The winter of 1947 was exceptionally cold, with air frost on 46 days during February and March. In comparison with the corresponding months in 1948 and 1949, mean monthly temperatures show that February, 1947, was colder by 9.7° F. and 11.5° F., and March, 1947, by 10.5° F. and 6.2° F. respectively. In 1947 the rainfall was above normal and the sunshine below normal for the whole period from March to July. In 1948, May was a comparatively dry month, but June and August were exceptionally wet and sunless. The summer of 1949 was exceptionally dry, with the sunshine well above normal from May to September inclusive. While it is difficult to draw any very definite conclusions on the influence of weather on worm egg output, the data in Graph 1 suggest that following bad weather—excessive rain or snow—the worm egg output tends to be higher than the average for that time of the year, and after good weather it tends to be below the average.

Discussion.

The results obtained from a study of nearly 1,500 hill sheep of the Blackface and Cheviot breeds in various districts of Scotland show that the seasonal pattern of the helminth egg output is very clearly defined, particularly in the case of adult sheep. From a low level in winter there is a marked increase in the spring to a peak about the end of May and early June. Following this, there is a fall towards the end of the year. Young sheep on the other hand, during their first year or so of life on the hill, show greater variability and, being more susceptible, give higher counts than older sheep. Further work is required before a clear picture is obtained of the rate of increase in worm infestation in hill lambs from birth until they are about six months old and termed "hoggs." It is probable, however, that there is a more or less gradual increase from birth in April until about early

Autumn, frequently followed by a fall towards the end of the year. The increase in the following spring, when the hoggs are about a year old, is often very high, although on one farm the hoggs showed a high output of eggs in January, followed by a gradual fall to a low level in June. It would seem therefore, that the hoggs have not yet reached that stability in their resistance to helminths which is evident in the majority of older sheep and that the seasonal pattern of egg output is therefore less clearly defined. It is also probable that they are more readily influenced by adverse conditions.

The first winter of their lives on the hills of Scotland can be particularly severe on young sheep faced with adverse weather and a low level of nutrition ; it is for this reason that the hoggs are often wintered away on low ground farms. Furthermore it is among the hoggs during the first months of the year that " black scours "—a disease associated with heavy trichostrongylid infestations—is usually seen. A high egg count in these sheep even as early as January is therefore not surprising.

The number of flocks which could be sampled in this investigation represented only a fraction of the total on Scottish hills ; but they were widely scattered throughout the Southern half of Scotland, and were representative of varied conditions of climate and hill grazings. There is therefore no reason to suppose that the seasonal pattern of the egg output varies significantly in different localities. Indeed a study of the literature on the subject shows that a spring increase in egg counts occurs not only in different countries with widely different climates, but also under widely different methods of sheep managements.

Schmid (1933) in Germany, although he did not employ an egg counting technique, found that the highest percentage of ewes and yearlings showing Strongyle eggs in the faeces occurred in April and the lowest in September. He, unfortunately, did not continue his observations through to the winter months.

In this country the problem was studied by Taylor (1935) on 12 breeding ewes kept on the Experimental Farm at Weybridge. These sheep were kept indoors during February and March and for the remainder of the year were at pasture. Taylor concludes from his studies, which extended over two years, that " the number of eggs of trichostrongylid worms passed by breeding ewes is at its minimum during the winter months and rises to a peak in June from which it falls again to the end of the year." He further concludes that " the rise and fall in the numbers of eggs passed by the ewes does not appear to

synchronise with the rise and fall in the intake of infective larvae."

In the United States, Hawkins and his co-workers (1944) studied the normal course of nematode infestations in untreated ewes and lambs under natural conditions of flock management. Their work is of particular interest in that it shows the rise and fall in the egg counts of individual species commonly found in sheep. Their graph giving the total egg counts throughout the year shows very clearly that the lowest egg output occurs in February, rising at the beginning of March and reaching a peak between April and May. After that time there is a fall to a low level by the middle of July and this continues until the end of the year. It is of great interest that these results were obtained in sheep which were in barns throughout the winter and were not put to pasture until the 20th of May. The spring increase therefore occurred before the sheep went to pasture. Similar results were obtained in 1947 by Hawkins and de Freitas on sheep wintered in barns. In experiments carried out by Seghetti and Marsh (1945) sheep were wintered and fed in paddocks which were mostly covered with snow during the early months of the year. The results in this instance were of interest in that the spring increase in egg output was not prevented by dosing with phenothiazine. This had also been recorded by Hawkins *et al.* in their second paper in 1944, but the peak did not reach the same height as that in the undosed control group. Naerland's (1949) observations in Norway also show the spring increase in eggs in in-wintered hoggs which were kept at different nutritional levels and in some instances even after anthelmintic treatment. He concludes that the increase was due to greater egg production in the female worms and, " Probably to a certain extent, to new females having developed to maturity from larvae which since the foregoing pasture season have been latently in the intestinal mucosa."

Extensive studies on the epidemiology of helminths in sheep have been carried out by Gordon (1948) in Australia. It is difficult, however, to compare the Australian results with those given in this paper, since certain species, such as *Oesophagostomum columbianum*, do not occur in this country, and *Haemonchus contortus* is of much greater importance than it is on Scottish hills. However, in Australia the heavy infestations with *Trichostrongylus* spp. in the winter may be compared to the outbreaks of winter trichostrongylosis in hill hoggs in Scotland.

Similarly Tetley's (1941 and 1949) detailed studies on the helminths of sheep in the North Island of New Zealand were made under very different conditions of climate, nutrition and stock concentration from those existing on Scottish hills.

In this country apart from the studies made by Morgan and Sloan (1947), which showed the pattern of egg output in the same flocks over a period of two years, the work on one age group (18 months old at the beginning of the experiment) by Cushnie and White (1948) is of interest. These workers were able to carry out weekly egg counts on a group of 50 sheep and the results therefore showed more accurately when the increase in egg counts began. It is seen from their graph that there was a slight upward tendency even in February and this became very pronounced during March. They concluded that the sheep could not have picked up new infestations during this period since the ground was covered with snow and the sheep had therefore no access to pasture.

It may be concluded therefore from all these observations that the spring increase in worm egg output in sheep is of wide occurrence and that it shows itself with surprising persistence under widely different methods of sheep management and climatic conditions.

It is not proposed in this paper to discuss the question whether the spring increase is due to increased egg production in worms which have been in the host throughout the winter, as young stages in the mucosa or as adults in the lumen of the alimentary canal as suggested by several authors, or whether it is due to new infestations picked up in the early months of the year. Data on this problem will be given in a later paper.

Summary.

From the study of the worm egg output of nearly fifteen hundred sheep on many hill farms in Scotland it has been shown that :—

(i) Worm egg output in ewes and gimmers is lowest in early winter and highest in spring and early summer ; this is followed by a marked decrease towards the winter months.

(ii) Generally in ewe hoggs the worm egg output is the same as that in older sheep, but shows greater variability ; on occasion the spring peak may be absent.

(iii) The level of worm egg output of hoggs is generally higher than that of older sheep, and in this respect gimmers tend to fall between hoggs and ewes.

(iv) Weather conditions may have an influence on the height and duration of the spring peak of worm egg production.

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Experiments with D-D Mixture Against Root-Knot Eelworm, *Heterodera marioni* (Cornu).

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In 1943, it was reported by Carter that D–D mixture had given good control of the Root-Knot Eelworm, *Heterodera marioni* (Cornu), in pineapple plantations in Hawaii where it had been tested over a period of three years under tropical conditions. In view of these promising results and those obtained by other American workers, notably Pinckard (1943) and Parris (1945), it was decided by the writer to test its value against the Root-Knot Eelworm under the conditions in the British Isles. This mixture is a dark-coloured, volatile liquid containing mainly 1–3–dichloropropylene and 1–2–dichloropropane with traces of higher chlorides. The experiments were carried out in 1947 in a commercial glasshouse at Bridgend, Glamorgan, South Wales, where tomato crops in previous years had produced poor yields of fruit due to heavy infestations of their roots by the Root-Knot Eelworm. This eelworm was the only parasitic nematode present and no serious attacks by any fungi or insects were observed during 1945–47, inclusive. The soil was a light loam in a high state of fertility with a fairly high content of organic matter, and excellent crops of tomatoes had always been grown in it prior to the establishment of the Root-Knot Eelworm in the soil. For many years, three crops per annum had been grown in this glasshouse, tomatoes from early April to mid-October, chrysanthemums from the latter part of October to about the end of January, and lettuce together with a variety of flowering plants in the intervening period between the chrysanthemums and the tomatoes. Of the crops grown in it, only the tomatoes were severely attacked each year, judging by the galled condition of the roots and stunted growth of the aerial parts of the plants.

General Details of the Experiments.

Four dosage rates of D–D, including zero, were tested on plots of equal dimensions on each side of a glasshouse which measured 100 feet

long and 20 feet wide :—
Plots E1 and W1—Untreated controls, no injection of D–D.
Plots E2 and W2—D–D applied at the rate of 200 lb. per acre.
Plots E3 and W3—D–D applied at the rate of 300 lb. per acre.
Plots E4 and W4—D–D applied at the rate of 400 lb. per acre.

The D–D mixture was applied by injection into the soil with a hand-operated applicator designed to deliver a pre-determined quantity per single stroke of the plunger. All the injections were made at a depth of seven inches and at points of fifteen inches apart in either direction. The actual points of injections were staggered to ensure even distribution of D–D throughout the soil. The quantities of D–D injected at this spacing to give the dosage selected were as follows :—

2.9 c.c. per injection = 200 lb. per acre.
4.4 c.c. per injection = 300 lb. per acre.
5.8 c.c. per injection = 400 lb. per acre.

When treated with D–D the soil was already well broken up and in excellent tilth to a good depth and it contained suitable moisture content for planting out tomatoes. These precautions were taken since soils which are too wet or too dry for easy working or in a poor state of cultivation do not permit proper diffusion of the vapours of D–D. Immediately after injection, all the plots were watered in such a way as to wet the soil evenly to a depth of one inch and also covered with damp sacks with a view to reduce the rate of volatilization of D–D. These sacks were kept moist for a period of fourteen days for the same purpose. They were then removed and during the next seven days the soil was forked on two separate occasions to encourage the disappearance of the fumigant from the surface layers of the soil in preparation for planting out the tomato seedlings. Both the treated and control plots were planted with tomato seedlings raised in sterilised soil, on 22nd April, 1947, some three weeks after the application of the D–D. Normal practice adopted under commercial conditions for the growing of tomatoes was followed in the cultivations and application of organic and inorganic manures.

Observations on Growth of Crop.

The tomato plants on all plots, including the controls, were examined at frequent intervals throughout the season when special attention was given to (*a*) signs of any pathological conditions on the aerial parts of the plants, (*b*) the rate of growth made by the plants, (*c*) the development of flower trusses and setting of the fruit, (*d*) yield of marketable tomatoes, and finally, after lifting the roots carefully, (*e*) the condition

of the root system of individual plants.

Experience gained in the use of D–D in the U.S.A. has shown that the vapours given off by this mixture are toxic to plants and that it is, therefore, necessary to allow an interval between injection and planting to safeguard against phytocidal effects. This interval varies from a few days to a few weeks, depending on the crop and local conditions, particularly temperature. Despite the fact that only an interval of three weeks elapsed in the present experiments between the application of D–D and planting out the tomato seedlings, no evidence of any phytocidal action on the plants was detected on any plots.

At first, the tomato plants on both the treated and untreated plots made remarkably good progress due undoubtedly to the cultural technique adopted by the grower. Towards the end of June a slight difference became apparent between the plants on the D–D treated plots and those on the controls. The tomato plants on all the treated plots at this stage, irrespective of dosage rates of D–D, were exceedingly even in growth and growing vigorously whereas those on the untreated plots appeared somewhat spindly in growth and unable to withstand the high temperatures around mid-day without exhibiting signs of wilting. As the season advanced, these differences in appearance of the foliage and reaction to high temperatures became more striking and in turn, in the case of the plants on the untreated plots, more typical of a severe root invasion by the Root-Knot Eelworm.

The treatment effects were reflected not only in more luxuriant growth and ability of the plants to cope with intense evaporation without showing signs of flagging of the leaves but also in a deeper green colour of the foliage as well as in production of larger flower trusses and, particularly, in better setting and earlier ripening of the fruit. Further, the general appearance of the plants on the untreated plots when compared with those on the controls strongly indicated that D–D had produced, in addition to nematocidal action, a soil-amendment effect somewhat similar to that of partial soil-sterilisation normally associated with the application of steam or certain volatile substances, such as formaldehyde or carbon bisulphide. Carter (1943, 1945), working with pineapple in Hawaii, claimed a soil-amendment effect from D–D, in addition to nematocidal action, and Peters (1948) has demonstrated, in experiments with D–D against *Heterodera rostochiensis*, that the former influence can be produced by this chemical substance, even in the absence of eelworm.

The main experimental effect was the contrast between the injected and control plots but about mid-August the plots also showed for the

first time in the season definite treatment differences. Evenness in height of the plants, luxuriance of their growth, size of their flower trusses and degree of setting of their fruit over the individual plots constituted the chief criteria of differences between the various D–D treated plots. When the performance of all the tomato plants taken together as a single unit on each plot was considered, the improvements were in direct relation to increases in dosage rates but some individual plants on all plots were outstanding and indistinguishable from one another, irrespective of the quantity of D–D which had been injected earlier in the season.

Picking of ripe tomatoes was commenced on all the treated plots about mid-July, but not on the controls until the second week in August and even then only on some 30 per cent. of the plants on each plot. The dearth of fruit remained one of the main characteristic features of the untreated plots throughout the season. This was attributable to the fact that no saleable tomatoes were produced at any time on the remaining 70 per cent. of the plants on either plots. Poorly developed flower trusses did appear on these plants but the few fruit that set on some of them failed to attain marketable size.

Apart from the pathological conditions symptomatic of root invasion by the Root-Knot Eelworm, no evidence of any disease was detected on the aerial parts of the plants on any plot until the end of September when a light infection by the fungus, *Cladosporium*, developed on the foliage of some plants on all plots.

Effects on Growth and Yield.

Of the plant criteria under examination, it was only possible to obtain actual measurements for the (*a*) number of marketable tomatoes produced by the plants on their third and fourth trusses, (*b*) heights attained by the aerial parts of the plants at full growth and (*c*) number of fruit-bearing trusses produced by the plants during the entire season. When measurements were made of these three factors chosen as criteria of efficiency, the extreme row on each side of every plot was discarded as a safeguard against the inclusion of any border influences resulting from inter-plot diffusion of D–D.

(*a*) *Yield of tomatoes.*—As expected in the case of trials carried out in a glasshouse devoted to the culture of tomatoes under commercial conditions, it was, unfortunately, not possible to obtain records of the weight of all the ripe fruit harvested from each plot over the whole season. In order to get some criterion of efficiency, based on yield, it was decided under the circumstances to record the number of market-

able tomatoes present on the third and fourth trusses of all the plants on each plot, including the controls. Table I, column 5, shows the mean number of tomatoes per truss under the different dosage rates of D–D and based on the numbers of saleable fruit recorded in this manner. It will be noticed that, in general, the tomato plants on the west side of the glasshouse made better growth (Table I, columns 3 and 4) and gave higher yields (column 5). On examination of the roots of the tomato plants at the end of the season (Table II) it was found, as expected from the results of an inspection of the tomato crop in the preceding year, that the east side (Plots E) was more heavily infested with the Root-Knot Eelworm than the west side (Plots W).

TABLE I.

Effects produced by D–D on growth and yield of tomato plants grown in soil heavily infested with the Root-Knot Eelworm, *Heterodera marioni* (Cornu).

Plot Index	*D–D lb./acre*	*Mean measurements*		
		Height of plants	*No. of trusses*	*Yield/ truss*
		Ft. in.	No.	No.
E1	0	7 1	6.8	2.7
W1	0	7 3	7.2	3.2
Average		7 2	7.0	2.9
E2	200	8 0	7.9	5.5
W2	200	8 3	8.2	5.9
Average		8 2	8.1	5.7
E3	300	8 7	8.9	9.7
W3	300	8 5	8.6	10.3
Average		8 6	8.8	10.0
E4	400	9 1	9.5	12.5
W4	400	9 1	9.4	12.2
Average		9 1	9.5	12.4

It will also be noticed in Table I, column 5, that the yields on the control plots were extremely poor, being rather less than about 25 per cent. of the normal crop produced under good commercial conditions. When it is taken into consideration, however, that no marketable

tomatoes were produced by about 70 per cent. of the plants growing on the control plots, it is evident that the remainder gave a fairly satisfactory yield compared with that obtained on the plots injected at the rate of 400 lb. of D–D per acre and where all the plants remained throughout the season indistinguishable from one another from the viewpoint of fruit production.

It will also be noted that there was on the plots treated with D-D at the rate of 200, 300 and 400 lb. per acre a graduated response at different rates of injection. The yields at the three dosage levels were respectively 197, 345 and 462 per cent. higher than in the controls. On critical examination it was found that these marked benefits derived from the increased quantities of D–D were associated not with the improved performance of the plants in general at any dosage rate but rather with the ratio of plants carrying heavy crops of tomatoes to those with little or no fruit of marketable size. All the plants on the plots injected at the rate of 400 lb. to the acre belonged to the former category, whereas only about 75 per cent. and 50 per cent. could be so classified on the plots treated with 300 lb. and 200 lb. dosage rates, respectively. The plants carrying good crops of tomatoes were exceedingly uniform in respect of number and size of tomatoes at all dosage levels. Those relegated to the unsatisfactory class, on the other hand, were extremely variable in these respects on all plots, but even the best of them seldom yielded more than 8 per cent. marketable fruit compared with that produced by the better type of plants on the same plot.

(*b*) *Height of plants.*—The maximum height attained by each plant, to the nearest inch, was measured, on 8th October, when the plants had reached their full growth. Table I, column 3, gives the mean heights of the plants for the four dosage rates, including zero. It will be seen that, on an average basis, there was an appreciable difference in height between the plants on the treated plots and those on the controls. Further, the height of the tomato plants on the D–D treated plots showed an increase in proportion to the rate of application within the range tested, being at the three dosage levels, 12, 16 and 23 inches, respectively, higher than those on the untreated plots. It must be pointed out that this criterion of efficiency of the various treatments proved rather unsatisfactory as it gave no true indication of the comparative amount of foliage growth produced by the tomato plants but it had the obvious advantage of ease of computation. The difference in the relative extent of foliage growth made by the plants under the various treatments were far more outstanding than the figures for the height measurements indicated. As in the case of the

yield criterion of efficiency, the difference in mean heights of the plants were related, though less pronounced, to the ratio of the two fairly distinct groups of plants to one another on each of the 0, 200, 300 and 400 lb. D–D designated plots rather than to comparatively small variations among the plants in general. One group comprised poor, stunted plants and the other, plants normal in appearance, the ratio of the former to the latter being approximately 75 : 25, 50 : 50, 25 : 75 and 0 : 100 for the 0, 200, 300 and 400 lb. dosage rates, respectively.

(*c*) *Number of flower trusses*.—The number of flower trusses produced by the plants during the entire season was recorded in early October. The mean numbers of trusses per tomato plant are presented by dosage rates in Table I, column 4. It will be observed that the number of trusses produced by the plants were quite satisfactory except by those on the controls and that increased dosages of D–D to the plots led to progressively greater numbers of trusses. The figures for the mean numbers of trusses, however, did not give a true reflection of the fruit potentiality of the plants, particularly on the control plots where all the flowers on the trusses on some 70 per cent. of the plants were virtually abortive. This also applied, but to a much lesser degree, to about 50 per cent. and 20 per cent. of the tomato plants on the plots treated with D–D at the rate of 200 lb. and 300 lb. per acre, respectively. In contrast, all the trusses on the most heavily treated plots (400 lb.) carried splendid crops of tomatoes.

NEMATOCIDAL EFFECTS.

Since chemical treatment of the soil can lead directly to an improvement in the growth of plants and in turn to an increase in the yields of the crops harvested from them without eelworms having been destroyed, it was decided to measure nematocidal effects independently. Accordingly, the stems of the plants on all plots were cut off at ground level and then the root system of each one of them was carefully lifted and cleared of all its adhering soil. Afterwards, the roots were critically examined for the abnormal swellings or galls associated with the presence of the Root-Knot Eelworm and as these were confluent with one another in the case of the more severely attacked plants, no attempt was made to count the number of separate galls on each root system. Instead, the roots of the plants on each plot were graded into four categories :—

1. Roots free of any symptoms of infestation.
2. Roots with light infestations, that is, roots with one to six small swellings.

3. Roots with moderate infestations, that is, roots with more than six small swellings but still with an appreciable amount of rootlets useful to the plant and free of any obvious signs of attack.
4. Roots with severe infestations, that is, roots completely deformed into swollen, entangled masses virtually useless to support any plant growth.

TABLE II.

Nematocidal effects produced by D–D on tomatoes grown in soil heavily infested with the Root-Knot Eelworm, *Heterodera marioni* (Cornu).

*Plot Index**	*D–D lb./acre*	*Degree of infestation of the roots (per cent.)*			
		Severe	*Moderate*	*Light*	*Free*
E1	0	72.7	18.2	9.1	0
W1	0	66.0	11.8	22.2	0
Average		69.4	15.0	15.7	0
E2	200	51.8	40.7	7.4	0
W2	200	45.1	33.3	16.7	4.9
Average		48.5	37.0	12.1	2.5
E3	300	17.6	35.4	35.2	11.8
W3	300	12.5	37.6	36.8	13.1
Average		15.1	36.5	36.0	12.5
E4	400	0	12.5	62.5	25.0
W4	400	0.9	40.1	36.4	22.6
Average		0.5	26.3	49.5	23.8

* Plots indexed E = east side of glasshouse; Plots W = west side.

The results obtained in this way are summarised, on a percentage basis, in Table II. It is evident from these results that the plots on the east side of the glasshouse (Plots E) harboured a heavier infestation than those on the west side (Plots W) and that of the D–D dosages tested, the best control of the Root-Knot Eelworm was obtained on the plots injected at the rate of 400 lb. to the acre. In both plots treated

at this rate of D–D, about 24 per cent. of the plants were free of infection and a large proportion of the remainder not badly infested, judging by the condition of the roots. In one of these plots, no roots were heavily infested and in the other, only 0.9 per cent. of them came within this category. This was a great improvement over the conditions on the

TABLE III.

Nematocidal effects produced by D–D on tomatoes grown in soil heavily infested with the Root-Knot Eelworm, *Heterodera marioni* (Cornu).

Plot Index	*D–D lb./acre*	*Degree of usefulness of roots for plant growth*			
		Virtually useless	*Moderately useful*	*Excellent*	*Useful**
E1	0	72.7	27.3	0	27.3
W1	0	66.0	34.0	0	34.0
Average		69.4	30.7	0	30.7
E2	200	51.8	48.2	0	48.2
W2	200	45.1	50.0	4.9	54.9
Average		48.5	49.1	2.5	51.6
E3	300	17.6	70.6	11.8	82.4
W3	300	12.5	74.4	13.1	87.5
Average		15.1	72.5	12.5	82.0
E4	400	0	75.0	25.0	100.0
W4	400	0.9	68.2	30.9	99.1
Average		0.5	71.6	28.0	99.8

* Figures in this column = total for columns 4 and 5 in Table III and columns 4, 5 and 6 in Table II.

control plots where no plants were free of infection and, on an average basis for the two plots, about 70 per cent. of them showed a severe infestation. Both the other rates of application of D–D produced a reduction in the intensity of attack, but to a lesser extent than that of 400 lb. per acre,

Taking all the data into account, it appears certain, therefore, that D–D reduced the infestation of the Root-Knot Eelworm under the conditions of these particular experiments, the reduction being roughly proportional to the dosage rate.

As measured by the ratio of useful roots for supporting plant growth, shown in Table III, column 6, it decreased the eelworm population by

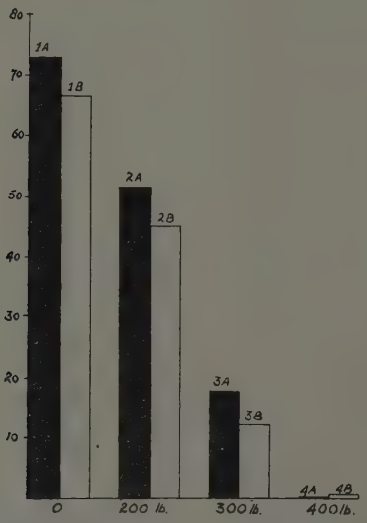

FIG 1:- PERCENTAGE OF TOMATO ROOTS HEAVILY INFESTED BY HETERODERA MARIONI (CORNU) IN SOILS INJECTED WITH D-D AT DOSAGE RATES OF 0, 200, 300, 400 lb PER ACRE.

something like 25 per cent., 50 per cent. and 75 per cent., when injected at the rate of 200, 300 and 400 lb. per acre, respectively. That such a classification of the roots into (*a*) roots virtually useless for all the needs of the tomato plant due to heavy infestation by *Heterodera marioni* and (*b*) roots moderately useful to satisfactory for all plant requirements seems to be justified. As already mentioned, it was found

that when the plots were examined, treatment by treatment, the plants on each plot fell naturally into two groups: (*a*) plants stunted and uneven in growth, unable to withstand high temperatures around noon without flagging, poorly developed flower trusses and little or no tomatoes of marketable size, and (*b*) plants uniform in size and appearance, luxuriant growth of foliage, well developed flower trusses

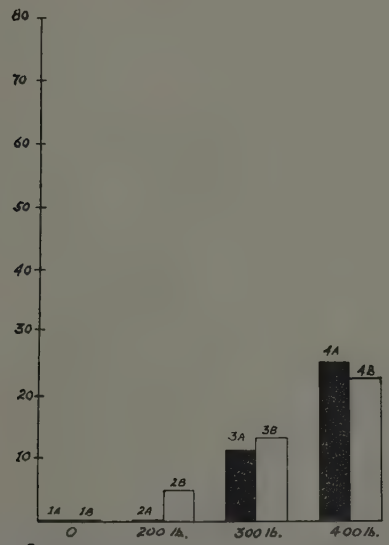

FIG 2:- PERCENTAGES OF TOMATO ROOTS FREE OF ATTACK FROM HETERODERA MARIONI (COARU) IN SOILS INJECTED WITH D-D AT DOSAGE RATES OF 0, 200, 300, 400 lb PER ACRE.

and splendid crops of tomatoes. The proportion of the latter type to the total number of plants under the 0, 200, 300 and 400 lb. dosage rates, on a percentage basis, roughly amounted to 25, 50, 75 and 100, respectively. These figures for the apparently healthy plants approximate remarkably closely to those for the roots considered useful for normal plant growth (Table III, column 6), that is, roots not heavily

infested by the Root-Knot Eelworm (Table II, columns 4, 5 and 6).

The beneficial effects produced by D–D on the root systems of the plants under the various dosage rates are depicted histographically in Figs. 1 and 2. It will be noticed that injections at the rates of 200, 300 and 400 lb. per acre reduced the proportion of heavily infested roots from about 70 per cent. on the control plots to 48.5 per cent., 15.1 per cent. and 0.5 per cent., respectively. Further, that these doses of D–D increased the proportion of unattacked roots, at least roots not obviously infected, from zero on the untreated plots to 2.5 per cent., 12.5 per cent., and 23.8 per cent., respectively.

Judging by the results obtained in the present experiments, the prospects for controlling the Root-Knot Eelworm under glass in the British Isles by injecting D–D into the infested soil are promising. No information regarding the quantity necessary to effect complete eradication is available but it seems that an application exceeding 400 lb. per acre would be essential for this purpose, at least in heavily affected soils where 70 per cent. of the plants are liable to suffer severe infestations. It would appear, on the other hand, that in such seriously infested soils, a dosage of 400 lb. to the acre is adequate to enable the grower to obtain a full crop of tomatoes.

Taint in Tomatoes.

It has been recorded by Peters (1949) that tomatoes grown in soil previously injected with D–D developed a pronounced and unpleasant taint in the fruit. No such evidence was obtained in tasting tests carried out with tomatoes grown in plots injected at the rate of 400 lb. per acre in the present experiments. Duplicate lots of tomatoes, designated x and y, were issued about mid-August to six persons with the information that one lot came from plants grown in soil which had received a special treatment. No taint was detected by any of them. The test was repeated with six different persons towards the end of September with the same result.

It is difficult to explain why there was no evidence in these tests of the objectionable taint discovered in experiments conducted at St. Albans (Peters, 1949). The anomally is probably explicable in terms of dosages, soils or cultural methods. It is significant in this connection that in the course of earlier work by the writer with another soil fumigant against *Heterodera marioni*, an unpleasant taint developed in the fruit of the tomato. This taint ceased to be detectable in cases where the growers had watered the ground, sometime prior to planting

the tomatoes, to such an extent as to soak the soil to a considerable depth and actually cause an appreciable amount of flooding. Such a procedure is commonly practised by growers and indeed highly recommended where heavy dressings of muriate of potash (Potassium chloride) have been applied in the previous season.

Summary.

A D–D mixture was tested against the Root-Knot Eelworm, *Heterodera marioni* (Cornu), in a glasshouse devoted to the growing of tomatoes under commercial conditions in South Wales. The effects of four different rates of application, namely, 0, 200, 300 and 400 lb. per acre injected at a depth of seven inches at staggered points fifteen inches apart were investigated.

Improvements in growth of the tomatoes and reductions in the eelworm manifestations on the roots were produced in proportion to the increases in the dosage rate. Height of the plants, number of flower trusses and yield of fruit were selected for actual measurements of the effects produced on the growth of the tomato plant. All of them showed a graduated response to the injections at 200, 300 and 400 lb. per acre, the yields of marketable tomatoes, for example, being at the three dosage levels, respectively, about 200, 350 and 450 per cent. higher than in the controls.

The nematocidal effects were measured independently by grading the roots into four distinct categories, according to the severity of the pathological manifestations associated with the presence of *Heterodera marioni*. Judging by the results obtained in this way, D–D reduced the infestation by something like 25, 50 and 75 per cent. when injected at the rate of 200, 300 and 400 lb. per acre respectively. No information regarding the quantity necessary to effect complete extermination is available but it seems that an application exceeding 400 lb. would be imperative for this purpose at least in heavily infected soils where 70 per cent. of the plants are liable to suffer severe infestations.

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On Sterilizing Land Against Poultry Parasites.

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The substance methyl bromide has been used during the past few years to treat soil in greenhouses where eelworms have been prevalent, and has proved particularly useful against cysts of *Heterodera* spp., which attack tomatoes and other indoor plants. Considerable success has apparently followed this treatment. The possibility of using it against the resting stages of animal parasites seemed reasonable, and experiments have been carried out to test its efficiency against ova and oocysts of sundry poultry parasites.

Monoxenous helminth parasites are not of any great importance where the battery system is practised for the rearing of young birds, but they can be quite troublesome among poultry on free range, particularly where the area of available land is restricted. Furthermore, not all species of gallinaceous birds can be reared satisfactorily under the artificial conditions which occur in batteries. For instance, some game birds are notoriously difficult to rear except on free range, and parasitic disease may become a menace, for it is often necessary to use the same rearing field, because of its situation or its physical characteristics, year after year. There are also many farm birds, e.g. turkeys, which are regularly reared on pieces of land which cannot be usefully employed for other purposes, and which otherwise would be wasted. In the course of time such odd corners tend to become heavily infested with poultry parasites, and disease may be the cause of many fatalities. It is not only the helminth diseases that assume epidemic proportions ; the protozoan diseases also bank up and cause serious losses among young stock.

A field attached to the research laboratories of I.C.I. Ltd., at Burgate Manor, was allocated some 2½ years ago for the rearing of young stock. It was divided up into a number of pens, into which were put several different species of gallinaceous birds. Unfortunately, it was soon found to be heavily seeded down with ova and oocysts of all the commoner poultry parasites. Deaths occurred almost daily and stocks

were rapidly reduced. Enterohepatitis, coccidiosis and quail disease were the chief causes of loss and the affected birds all carried abnormally large numbers of *Heterakis gallinae* and *Ascaridia lineata*. The only cestode was *Davainea proglottina*, and this appeared first in bobwhite quail, *Colinus virginianus*, and was associated with the ulcers of quail disease. Later on there also appeared spontaneously *Trichostrongylus tenuis*, *Syngamus trachea* and various species of *Capillaria*. Chicken and pheasant proved very susceptible to caecal coccidiosis—the species concerned is probably *Eimeria tenella*—and though treatment with sulphamezathine was successfully carried out, yet this is costly and time consuming and treated birds are unsuitable for scientific work. Enterohepatitis attacked chicken, *Gallus gallus*, grey partridge, *Perdix perdix*, redleg partridge, *Caccabis rufa*, and pheasant, *Phasianus colchicus*, and was a continual source of loss. As it is necessary to have parasite-free chicks for experimentation, much consideration has been given to possible means of cleaning the land. Meanwhile birds are reared on wire floors, a method which precludes many parasitic diseases, but which introduces difficulties of its own, usually associated with diets.

Many chemical substances have been recommended from time to time for the destruction of parasitic eggs and ova. Lime for instance is widely used in the belief that it kills the infective stages of poultry parasites. It may have a good effect on the pH and fertility of the land ; it may encourage growth of a healthy flora, but in agricultural doses it has no significant effect on helminth ova, while coccidial cysts seem to be even more resistant.

One particular pen, 10 yards square, proved to be heavily infested with *Histomonas meleagridis*, *Eimeria tenella* and with the common poultry nematodes, and was therefore particularly suitable for trials. It formed part of a meadow which had not been ploughed or cultivated for many years. The turf consisted of the commoner grasses and some weeds. There were species of *Bromus*, *Lolium*, *Agropyron* and *Dactylis* among the grasses while the weeds consisted of plantain, some thistles, a few nettles and yarrow. Owing to its lack of cultivation the surface was rough and the grasses appeared as large irregular tussocks. For the experiment the long grass was cut roughly with a hand sickle before being treated with methyl bromide. A trench was dug round the perimeter, ten inches deep, the soil being thrown inwards. In the middle of the pen a small phial containing 25 gm. methyl bromide was placed in a pit 10 inches deep and lightly covered with soil. A short upright stick marked the spot. The whole surface was then covered

with rubberized balloon fabric, the edges of which were firmly embedded in sterile sand in the trenches on the boundary. The methyl bromide was released by breaking the phial with the marking stick. The balloon fabric was removed 24 hours later. Numbers of dead slugs, snails, and earthworms were found on the surface of the ground as well as some beetles and Orthoptera. Samples of turf and soil were examined from various depths. The insect fauna usually associated with the tussocks of coarse grass had been destroyed and soil samples from a depth of 9 inches showed no living invertebrata.

Ten bantam chicks, three days old, which had been hatched in an incubator were introduced on 5 July, 1948, to this land in a brooder warmed electrically. Faecal examinations, involving centrifugation in saturated saline, were carried out at three day intervals for the next 30 days. No helminth ova were ever found. At the 21st day, 26 July, 1948, a single oocyst of *Eimeria* sp. appeared. This was solitary, for no more were found though 25 gm. faeces were immediately examined by concentration methods. It may have occurred through faulty technique. These birds were destroyed on the 32nd day, 6 August, 1948, and proved to be completely negative for all parasites.

A week later, 12 August, 1948, six pheasant poults were introduced ; they were then 21 days old. They had been hatched in an incubator and reared on wire floors. They remained on this land for 31 days, faecal examinations being made twice weekly. No ova or cysts were found and at autopsy, 12 September, 1948, they too proved to be negative for parasites.

Grey partridges, *Perdix perdix*, were then introduced, 16 September, 1948. Only two were available for the test. They had been reared on wire floors and were 28 days old when introduced to the pen, where they remained for six weeks. Faecal examinations, made twice weekly, were negative for parasites, as were the birds when they were finally destroyed on 28 October, 1948.

The land was left fallow during the winter months. During this period no birds have been specifically introduced, but wild birds, mainly starlings, rooks, jays, magpies and chaffinches have been seen flying in and out. On 5 May, 1949, 12 chickens were introduced and allowed to remain there for 32 days until 9 June, 1949. Faecal examinations were made and nematode ova were isolated from them after 26 days. Post mortem examination showed that *Ascaridia lineata* and *Heterakis gallinae* were present in some of the birds. Only three *Ascaridia* were found, one in each of three birds, and they were all

Date	*Species*	*Age*	*No. of birds*	*Faecal examination*	*Destruction*	*P.M. finding*
5–7–48	*Gallus gallus*	3 days	10	1 oocyst of *E. tenella* on 21st day	6–8–48	Negative for all parasites
12–8–48	*Phasianus colchicus*	21 days	6	Negative	12–9–48	Negative
16–9–48	*Perdix perdix*	21 days	2	Negative	28–10–48	Negative
5–5–49	*Gallus gallus*	3 days	12	Ova of *H. gallinae* appeared on 26th day	9–6–49	No. 157 2 *Heterakis* 158 6 *Heterakis*, 1 *Ascaridia* 159 35 *Heterakis*, 1 *Ascaridia* 160 6 *Heterakis*, 1 *Ascaridia* 161 Negative 162 Negative 163 11 *Heterakis* 164 4 *Heterakis* 165 Negative 166 Negative

immature. Caecal worms were found in six birds, one having as many as 86 worms. Four of the birds contained no parasites.

The plot is surrounded on three sides by untreated plots which act as controls. They have been occupied continuously by various species of birds—chickens, pheasants and partridges. All carry parasites, and deaths from parasitic disease occur at frequent intervals—enterohepatitis and coccidiosis have been particularly prevalent and the grey partridge has been extremely susceptible to both these diseases. Other grey partridges have died from strongylosis—2,006 *T. tenuis* being the largest number of worms found in any bird. These birds have also carried heavy incidental infestations of the common helminths—*H. gallinae, A. lineata, C. longicollis,* as well as light infestations of *S. trachea* and *Davainea proglottina.* There seems no doubt therefore that the gassed pen was completely free from parasites and parasite-borne disease for at least four months, and that even after a year there has been very little re-introduction. The destruction of the ova of *H. gallinae* has meant the disappearance of *Histomonas meleagridis* and therefore of enterohepatitis. That the methyl bromide has been the agent responsible for the destruction of ova and oocysts seems to be a reasonable conclusion. Visitors have been discouraged from entering this experimental plot but otherwise no particular precautions have been taken to prevent re-infestation. It was inevitable that this would occur, for wild birds and wind are natural disseminators of ova and oocysts. A period of four months is long enough for gamekeepers to rear their young stock and bring them to an age when they have developed considerable resistance to the more serious effects of parasitism, so it would seem that a single treatment would give complete protection to the poults hatched one year, and a large measure of protection to those hatched the following year.

LABORATORY EXPERIMENTS.

Tests have been carried out specifically with ova and oocysts *in vitro.* A plot of land 10 yards square was converted into a gas tight chamber in which were deposited sundry Petri dishes which contained ova and oocysts as follows :—

1. Embryonated ova of *Ascaridia lineata* which had been passed normally in faeces and had developed to the infective stage in the laboratory.

2. Freshly passed ova of *A. lineata* in faeces. These had been passed the previous day and had segmented only to the 32–64 celled stage.

3. Teased up adult *A. lineata* worms from which were released unembryonated ova, together with a large number of immature forms.

4. Embryonated ova of *Heterakis gallinae* obtained from adult worms and developed in the laboratory.

5. Non-embryonated ova of *H. gallinae* obtained from adult worms.

6. Embryonated ova of *Syngamus trachea* obtained from adult worms and containing actively moving larvae.

7. Non-embryonated ova of *S. trachea.*

8. Earthworms (*Lumbricus terrestris*) containing encysted gapeworm larvae.

9. Gravid segments of *Choanotaenia infundibulum.*

10. Oocysts of *Eimeria tenella* from the caecum of a pheasant.

These Petri dishes were placed in pits about 4 inches below the general surface of the ground and 25 gm. methyl bromide was used to fumigate the chamber, the cover being removed after 24 hours.

When examined under a microscope the larvae of *A. lineata*, *H. gallinae* and *S. trachea* appeared to be destroyed. There was no movement within the egg shell and none could be induced by gentle heat. They were fed to young chicks, 10 days old, but no infestations resulted. At autopsy the birds were completely clean of all parasites. Control chicks were fed untreated ova from the same source. From doses of 200 ova each there were developed mild infestations of 11, 5 and 6 *A. lineata* after one month. Similar feedings of 200 *H. gallinae* untreated ova resulted in infestations of 17, 23 and 19 worms in the caeca of the three birds.

The non-embryonated ova were kept at laboratory temperatures for six weeks but no development occurred. The contents rapidly broke down and oil globules appeared within the egg shell. No larvae developed.

The earthworms containing encysted gapeworm larvae were dead. As it was not practical at that moment to search for larvae within the body musculature, the worms were fed intact to chicks three days old, but none gaped and none carried any worms when they were examined post-mortem. The larvae must have been destroyed at the same time as the earthworms.

The gravid segments of *Choanotaenia infundibulum* did not appear to be materially changed by the action of the bromide and the hexacanth embryos had a normal appearance. Adult *Musca domestica* and *Lucilia* sp. were allowed access to this material and readily deposited ova upon it. These hatched and the resulting larvae fed voraciously. Some were allowed to metamorphose. Both larvae and imagos were

fed to chicks without result however. This is not conclusive for no encysted larvae were found in the few larvae and imagos that were examined by dissection. *Musca domestica* is a proved vector of *C. infundibulum* and *Lucilia* sp. is a possible one.

The oocysts of *Eimeria tenella* did not appear to be changed by the treatment but they produced no infections when fed to young chicks though this particular strain is quite virulent to chicks under natural conditions.

These *in vitro* tests suggest that methyl bromide has a strong lethal action on ova and oocysts of poultry parasites. The evidence from them and from the field test already described gives promise of a possible method of cleaning up reasonably small areas of land. The technique already described is lengthy and therefore expensive, but satisfactory gas tight chambers have been obtained on other plots by simply opening up the soil with a spade, tucking in the edge of the material and allowing the soil to fall back into position. This takes much less time, but experiments are still being made to reduce costs and time much more.

I am indebted to Dr. Ivan Parnell of the University of Edinburgh, who has much experience on the control of helminth ova by chemical means, for some very interesting and useful discussions.

SUMMARY.

Tests have been made with methyl bromide to investigate its efficiency in destroying infective stages of some helminth and protozoan parasites of poultry and game birds. It appears to have an immediate strong lethal action against ova of the common nematode parasites and against oocysts of *Eimeria tenella.* Its efficiency against ova of *Choanotaenia infundibulum* was not definitely proved. When used in the field, it penetrates soil to a depth of several inches, and in so doing destroys both ova and many invertebrates known to be vectors of poultry parasites. Land which has been treated remained quite free from parasitic infection for many months but gradually became re-infested. Wild birds were probably the agents for they carry ova accidentally on their feet and in the gut.

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Observations and Experiments on Stem Eelworm, *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936, with Special Reference to Weed Hosts.

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It is well known that certain weeds can act as alternative hosts for some races of stem eelworm, thereby tending to defeat the objects of crop rotation. During the course of advisory work in this province, collections of weeds have been made from various sites where stem eelworm attacks were known to have occurred, with a view to discovering which ones were likely to act as bridging hosts.

While it is fully realised that the presence of *D. dipsaci* in a weed growing amongst a crop infested with this eelworm does not prove that the eelworms are of the same biologic race, it is felt that observations of this kind are a valuable and necessary preliminary to host transference experiments.

The various records obtained have accordingly been collected together and are set out below under the headings of the crops concerned.

Note on Technique.

Baermann funnels were used in examining the weeds for the presence of *D. dipsaci*. After removing any roots, the weeds were thoroughly washed and cut up with scissors, selecting especially the lower parts of stems and petioles. Little significance has been attached to very small numbers of *D. dipsaci* from a given plant unless results were consistent for several fields.

A.—Agricultural Crops.

(1) *Red Clover.*—Weeds have been examined from three fields in Somerset and one in Gloucestershire. Results of examination are shown in Table I.

Trefoil (*Medicago lupulina* L.) was included in the seed mixtures used for the leys, and plants collected from all four fields yielded small numbers of *D. dipsaci:* no symptoms were observed.

(2) *Oats.*—*D. dipsaci* has frequently been found in the following three weeds taken from fields where oats have been attacked by stem eelworm :—

Common chickweed, *Stellaria media* (L.) Vill.
Mouse-ear chickweed, *Cerastium vulgatum* L.
Cleavers, *Galium Aparine* L.

These are already well known as hosts of the oat race of *D. dipsaci*. Scarlet pimpernel (*Anagallis arvensis* L.) and black bind-weed (*Polygonum Convolvulus* L.) have been recorded as hosts of *D. dipsaci* in association with infested oats, but little evidence has been obtained that this is the case in the South West. Devon cases of " tulip-root " occurred where pimpernel and black bind-weed were quite free from infestation.

Table I.

D. dipsaci present in numbers	Occasionally lightly infested. (Doubtful hosts.)	Free from *D. dipsaci*
Curled dock, *Rumex crispus* L. Light to moderate infestation in all four fields	Dandelion, *Taraxacum officinale*, L.	Sow-thistle, *Sonchus oleraceus* L.
	Groundsel, *Senecio vulgaris* L.	
	Smith's cress, *Lepidium Smithii*, Hook	Oxtongue Thistle, *Helminthia echioides* Gaertn.
Mouse-ear chickweed, *Cerastium vulgatum* L. Occurred in one field only, moderately infested	Forget-me-not, *Myosotis* sp.	Woolly thistle, *Carduus eriphorus* L.
	Fat hen, *Chenopodium album* L.	
	Cut-leaved cranesbill, *Geranium dissectum* L.	Buttercup, *Ranunculus* sp.
Cleavers or Goosegrass, *Galium Aparine* L. Present and infested in three fields	Ribwort plantain, *Plantago lanceolata* L.	Procumbent speedwell, *Veronica agrestis* L.
	Wall speedwell, *Veronica arvensis* L.	Greater plantain, *Plantago major* L.
	Common chickweed, *Stellaria media* (L.) Vill.	Hairy vetch, *Vicia hirsuta* Gray
	Scarlet pimpernel, *Anagallis arvensis* L.	Field Madder, *Sherardia arvensis* L.
		Coltsfoot, *Tussilago farfara* L.
		Knot-grass, *Polygonm aviculare* L.
		Orache, *Atriplex* sp.
		Hemlock, *Conium maculatum* L.

One of the present authors (L.N.S.) has recorded *D. dipsaci* in thyme-leaved sandwort (*Arenaria serphyllifolia* L.) in association with " tulip-rooted " oats in Devon. (Staniland, 1945).

Plants of wild oat (*Avena fatua* L.) were collected from a field in

Somerset where oats had been attacked by eelworm. These were showing striking " tulip-root " symptoms, i.e., swelling of the base and distortion of the tillers, and were found to be infested with *D. dipsaci*. The farm concerned had a bad reputation for eelworm trouble in oats. It seems likely that these wild oats, which occurred abundantly in many of the fields, have been a major factor in maintaining regular infestations.

Speedwell (*Veronica* sp.) and onion couch (*Arrhenatherum avenaceum* Beauv. var. *bulbosum* Lindl.), from the same field as the wild oat, showed no infestation.

(3) *Mangolds.*—Goodey has transferred *D. dipsaci* from diseased mangolds to oat seedlings and considers that the same biologic race attacks both crops. (Unpublished.)

In Spring, 1949, several cases of mangold rot due to stem eelworm came to the writers' notice. A collection of weeds from one of the affected fields, in Dorset, was examined for *D. dipsaci*. The results are shown in Table II :—

Table II.

Infested with *D. dipsaci*	Uninfested
Common chickweed, *Stellaria media* (L.) Vill.	Speedwell, *Veronica* sp.
Mouse-ear chickweed, *Cerastium vulgatum* L.	Greater Plantain, *Plantago major* L.
Cleavers, *Galium Aparine* L. Plants from the edge of the field only, lightly infested	Parsley piert, *Alchemilla arvensis* Scop. Silver weed, *Potentilla Anserina* L.
Dock, *Rumex crispus* L. Lightly infested.	Creeping buttercup, *Ranunculus repens* L. Shepherds purse, *Capsella bursa-pastoris* Medik Thistle, *Cirsium arvense* Scop. Scentless mayweed, *Matricaria inodora* L. Groundsel, *Senecio vulgaris* L. Annual meadow grass, *Poa annua* L.

At the time of sampling, the field was in wheat. Samples of this were also examined but yielded no *D. dipsaci*. A few were obtained, by Baermann extraction, from a small quantity of soil shaken from the roots of the weeds.

One of the writers (L.N.S.) found a heavy infestation of *D. dipsaci* in a sample of black bind-weed (*Polygonum Convolvulus* L.) from amongst young infested mangolds in a Devon field. These had followed a " tulip-root " oat crop. The record is felt to be of considerable interest as supporting the view that a race of *D. dipsaci* can attack both crops.

(4) *Fullers Teasel.* (*Dipsacus fullonum* L.).—In the Spring of 1949 Dr. Goodey, in company with one of the writers, collected a number of weeds from two eelworm-infested teasel fields in the Langport district of Somerset, and has kindly permitted us to include his results of examining these. (Table III, below) :—

TABLE III.

Field	Infested	Uninfested
No. 1	Mouse-ear chickweed, *Cerastium vulgatum* L. Creeping thistle, *Cirsium arvense* Scop. *D. dipsaci* present only in the outer dead leaves of the rosette.	Common chickweed, *Stellaria media* (L.) Vill. Cleavers, *Galium Aparine* L. Speedwell, *Veronica agrestis* L. Coltsfoot, *Tussilago farfara* L.
No. 2	Mouse-ear chickweed, Common chickweed, Cleavers.	Field madder, *Sherardia arvensis* L.

B.—Horticultural Crops.

(1) *Strawberry.*—Infestations of stem-eelworm were found to be widespread amongst strawberries in the Cheddar district during the Spring and Summer of 1949. There is evidence to suggest that the infestations may have originated from infested onions, and preliminary host transference experiments (see later) support the view that the biologic race concerned is the oat-onion race. Several cases were reported where well-defined patches of onion " bloat " had occurred in past years, corresponding in position with patches of *D. dipsaci* infestation in the strawberries.

Several collections of weeds were made in infested fields. Chickweed (*Stellaria media*) from several sites was found to be infested. In one case in particular, this plant showed symptoms suggestive of eelworm attack, the leaves being unusually thickened and waved.

Small numbers of single individuals of *D. dipsaci* were obtained, on one occasion, from the following weeds :—

Purple deadnettle, *Lamium purpureum* L. ; Greater plantain, *Plantago major* L. ; Procumbent speedwell, *Veronica agrestis* L.
The following were quite free from *D. dipsaci* :—

Cleavers, *Galium Aparine* L. ; Dandelion, *Taraxacum officinale* L. ; Ivy leaved speedwell, *Veronica hederaefolia* L. ; Bindweed, *Convolvulus arvensis* L. ; Groundsel, *Senecio vulgaris* L.

Of special interest was a small volunteer potato plant found growing close to an affected strawberry. This was showing symptoms of swelling in the stem and bases of the petioles and an examination showed considerable numbers of *D. dipsaci* to be present within the tissues of these parts. Search was later made on the same site for further potato plants showing similar symptoms. Only plants showing normal growth could be found, but a small number of these, when cut up into a Baermann funnel, yielded four adults of *D. dipsaci*. A small self-sown tomato plant from the same site showed no signs of infestation.

Most of the strawberries attacked by stem eelworm also contained *Aphelenchoides fragariae* in varying numbers. It is interesting to note that worms indistinguishable from this species* were obtained in small numbers from some of the above weeds, namely, cleavers, ivy-leaved speedwell, procumbent speedwell, and bindweed.

(2) *Phlox* (*Phlox paniculata*).—Weeds from a bed of eelworm infested phlox at Bath were collected and examined during summer 1949 by R. Sleigh. The results were as follows (Table IV) :—

TABLE IV.

D.dipsaci present in numbers	Lightly infested (Doubtful hosts)	Free from *D. dipsaci*
Shepherds purse, *Capsella bursa-pastoris* Medik Procumbent speedwell, *Veronica agrestis* L. Mayweed, *Matricaria* sp.	Chickweed, *Stellaria media* (L.) Vill. Groundsel, *Senecio vulgaris* L. Annual meadow-grass, *Poa annua* L.	Annual nettle, *Urtica urens* L. Goosefoot or Fat hen, *Chenopodium album* L.

A batch of weeds from an infested phlox bed in Bristol was examined in Autumn, 1949. One species only, hairy bittercress (*Cardamine hirsuta* L.), was found to be infested with *D. dipsaci*.

Uninfested weeds were :—

Wall lettuce, *Lactuca muralis* Gaertn. ; Groundsel, *Senecio vulgaris* L. ; Sow-thistle, *Sonchus oleraceus* L. ; Daisy, *Bellis perennis* L. ; Procumbent speedwell, *Veronica agrestis* L. ; Greater plantain, *Plantago major* L. ; Annual meadow grass, *Poa annua* L. ; Petty spurge, *Euphorbia Peplus* L.

* Nematodes, closely resembling *A. fragariae*, but quite distinct from the common non-parasitic *A. parietinus*, were on one occasion found by one of the writers (J. F. S.), on *Galium Aparine* and red clover plants from a *D. dipsaci*-infested clover ley. As far as is known, no strawberries had been grown on or anywhere near this land.

(3) *Narcissus*.—Various possible weed hosts of the narcissus race of *D. dipsaci* have been recorded in the past, notably the plantains, *Plantago lanceolata* L. and *P. maritima* L., cats-ear (*Hypochoeris radicata* L.), crow garlic (*Allium vineale* L.) (Hodson, 1931), and wild leek (*Allium triquetrum* L.) (Goodey, 1929).

In an endeavour to find other possible hosts, opportunity was taken over a number of years to examine weeds in heavily infested fields in Devon and Cornwall (L.N.S.), but little or nothing could be added to this list. The only case where a substantial infestation was found was in cleavers growing amongst heavily infested bulbs in a garden at Newton Abbot. The bed concerned had previously grown rose bushes following grass.

The following list of plants from heavily infested fields of bulbs have given negative results :—

Common chickweed, *Stellaria media* (L.) Vill ; Mouse-ear chickweed, *Cerastium vulgatum* L. ; Cleavers, *Galium Aparine* L. ; Speedwell, *Veronica* sp. ; Scarlet pimpernel, *Anagallis arvensis* L. ; Shepherds purse, *Capsella bursa-pastoris* Medik.; Groundsel, *Senecio vulgaris* L. ; Mayweed, *Matricaria* sp. ; Sow-thistle, *Sonchus oleraceus* L. ; Creeping thistle, *Cirsium arvense* Scop. ; Creeping buttercup, *Ranunculus repens* L. ; Forget-me-not, *Myosotis* sp. ; Wild pansy, *Viola arvensis* Murr. ; Fumitory, *Fumaria officinalis* L. ; Annual nettle, *Urtica urens* L. ; Annual meadow grass, *Poa annua* L. ; Potatoes (Ground keepers).

Host Transference Experiments.

Experiments have already been carried out by various workers confirming that stem eelworm infestation can pass from crop plants to certain weeds and *vice-versa*. It is hoped, as opportunity permits, to gain more information on these lines based on the foregoing host records. A few successful transferences have, however, been carried out between various crop plants, and the results of these are set out below.

Transference of D. dipsaci *from oats to strawberry*.—A light infestation of *D. dipsaci* has been set up in a healthy Royal Sovereign strawberry runner grown in a pot, using dried " tulip-root " oats as an inoculum, which was introduced into the soil around the crown and among the young leaves of the growing point region. The oat material was added towards the end of July, 1949, and crinkling and darkening of the laminae of several of the younger leaves, characteristic of stem eelworm attack, was noticeable by the second week in September. On teasing a leaflet in water several individuals of *D. dipsaci* were liberated from

the basal portions of the main veins. The rest of the leaves showing symptoms were found to be lightly infested by Baermann extraction.

Mr. J. B. Goodey informs the writers that he has successfully transferred infestation in the reverse direction, from strawberry to oat seedlings, the latter developing typical " tulip-root " symptoms.

Strawberry runners were at the same time grown in contact with dried infested material of onion, red clover and strawberry. No infestation has been found in any of these up to the time of writing, but no conclusions can be drawn from these negative results since *D. dipsaci* has failed to pass from infested to healthy strawberries.

Transference of D. dipsaci *from clover, teasel and strawberry to onion.*— Infestation of onion seedlings by *D. dipsaci*, together with symptoms of stunting and distortion, has been set up by inoculation with dried infested material of onion, strawberry, fullers teasel (flower heads) and red clover. The dried material was broken up and mixed into the top inch or so of soil in six-inch flower pots. Two pots were inoculated with the strawberry material and one with each of the others. Onion seed was treated with 0.025 per cent. Iodine in Potassium Iodide solution as a precaution against possible eelworm infection, washed in water and sown fairly thickly in each pot.

Three weeks after sowing, typical symptoms of *D. dipsaci* attack were visible on the seedlings in all five pots. On teasing up samples of the affected seedlings in water, numbers of adults eggs and larvae were liberated from the tissues.

The proportions of seedlings showing symptoms at this stage were roughly as follows :—

Inoculated with Onion	...	over 75 per cent		
,, ,, Strawberry		50	,, ,,	in both pots
,, ,, Teasel	...	over 75	,, ,,	
,, ,, Clover	...	25	,, ,,	

These proportions subsequently increased somewhat, although the onions infested from clover were much less severely affected than the others.

In order to check the possibility of any initial infection in the onion seed surviving the iodine treatment, two pots were later set up as controls without the addition of any infested material. Onion seed from the same packet as before was sown untreated in one pot and after iodine treatment in the other. Sample seedlings were examined one month after sowing and no infestation was found then or later.

The teasel-head material used in this experiment contained a good deal of seed. Much of this germinated alongside the onion seed. The

young teasel seedlings proved to be infested with *D. dipsaci* and staining with Sudan IV and Flemming's solution showed clearly the position of the worms in the tissues. They occurred chiefly in the cotyledons, although a few were seen in the hypocotyl and the first pair of true leaves.

Discussion and Conclusions.

Examination of weeds taken from fields where various agricultural and horticultural crops had been attacked by *Ditylenchus dipsaci* showed that certain of these harboured considerable numbers of this nematode, and it is considered that these are likely alternative hosts of the races of *D. dipsaci* concerned. In a few cases such observations have been confirmed by transference experiments but further work is needed.

There is evidence that the behaviour of some biologic races of stem eelworm may vary in different parts of the country, particularly in the case of the " oat race," where certain weeds recorded as hosts in the Eastern half of England do not appear to carry infestation in the South West.

(1) *The Red Clover Race.*—Three weeds have been noted as hosts of *D. dipsaci* in association with infested clover, namely curled dock (*Rumex crispus* L.), mouse-ear chickweed (*Cerastium vulgatum* L.) and cleavers (*Galium Aparine* L.). No transference experiments have as yet been carried out. It should, however, be added that cases of clover eelworm have been found where seed infection could be ruled out as source of the trouble, and where the presence of weed hosts during the interval between successive clover crops seems the only likely explanation.

D. dipsaci was successfully transferred from red clover to onion.

(2) *The Oat, Mangold and Strawberry Races.*—Successful transferences of *D. dipsaci* have already been carried out from common chickweed (*Stellaria media* (L.) Vill.) to oats and back to chickweed, and also from cleavers and sandwort (*Arenaria serpyllifolia* L.) to oats (Stanliland, 1945). Mouse-ear chickweed is also commonly infested amongst " tulip-rooted " oats, and wild oat (*Avena fatua* L.) was found infested and showing symptoms in one field where oats were known to have been attacked. Experiments are needed to confirm that *D. dipsaci* can pass from oats to these weeds and *vice-versa*.

As has already been indicated, there is fairly conclusive evidence that oats and mangolds are attacked by the same race of *D. dipsaci*. The fact that the three principal weeds generally regarded as hosts of

the oat race, namely common chickweed, mouse-ear chickweed and cleavers, have been found harbouring *D. dipsaci* amongst infested mangolds, is in agreement with this view. Docks (*Rumex crispus* L.) and black bind-weed (*Polygonum Convolvulus* L.) may also be listed as possible hosts of this race in the South West.

Fields of strawberries, attacked by *D. dipsaci* at Cheddar, Somerset, contained infested chickweed (*Stellaria media*). *D. dipsaci* was also obtained from "ground-keeper" potatoes from the same fields and these may be of importance as bridging hosts. Further observations on weeds and host transference experiments are needed.

D. dipsaci has been successfully transferred from oats to strawberry and from strawberry to onions (and strawberry to oats by J. B. Goodey), and it is considered that this strawberry race is probably identical with the common oat race, already known to attack onions.

(3) *The Teasel Race.*—Although use of infested seed is undoubtedly the main source of eelworm trouble in teasels (locally called "Cabbagy Plant") in the Langport district of Somerset, observations have indicated that weed infestation may also play a small part in the problem. Three weeds, namely common chickweed, mouse-ear chickweed and cleavers, have been found as hosts of *D. dipsaci* amongst infested teasels. It is of course possible that these became infested from oats, clover or other crops grown previously, and transference experiments are again needed to settle the matter.

Nothing is known of the host range of the race of *D. dipsaci* occurring in the Somerset teasles, beyond the fact that onions are attacked.

(4) *The Phlox Race.*—As a result of examining weeds from eelworm infested Phlox beds, the following are suggested as possible hosts of this race: shepherds purse (*Capsella bursa-pastoris* Medik.), procumbent speedwell (*Veronica agrestis* L.), mayweed (*Matricaria* sp.) and hairy bittercress (*Cardamine hirsuta* L.). No host transference experiments have as yet been carried out between phlox and these weeds.

(5) *The Narcissus Race.*—Hodson (1931) has shown that cross infection can sometimes take place between Narcissus and certain weeds, notably the plantains, *Plantago lanceolata* L. and *P. maritima* L. and cats-ear (*Hypochoeris radicata* L.). The situation appears to be complex and it is possible that more than one race is involved. Other weeds recorded as possible hosts have been *Allium vineale* L. (Hodson, 1931) and *A. triquetrum* L. (Goodey, 1929). As a result of observations by one of the writers, cleavers may be added to these.

Much more work is needed on the host relationships of the race or races of *D. dipsaci* attacking narcissus.

ACKNOWLEDGMENTS.

Grateful acknowledgments are due to Dr. T. Goodey and Mr. J. B. Goodey for permission to include results of certain unpublished work, and to the former for reading through the typescript. The writers are also greatly indebted to Dr. Goodey and his department for their ready co-operation and advice.

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*On the species of *Paramphistomum* Fischoeder, 1901 Occurring in Britain and Ireland with Notes on Some Material from the Netherlands and France.

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It has hitherto been assumed that the only paramphistome parasitising ruminants in Britain and Ireland is *Paramphistomum cervi* (Zeder, 1790) Fischoeder, 1901. The incidence is slight and only three records of its occurrence have been found in the literature. It was recorded by Pillers (1922) from a cow in Cheshire, by Craig and Davies (1937) from sheep in Cheshire and by Kelly (1948) from cattle in several districts in Ireland. There have also been a number of unconfirmed reports of its being found in various districts. Two veterinary surgeons state that they have found paramphistomes in the rumen of sheep in Herefordshire. The author has also been told of their being found in the rumen of a cow in this county and it was suggested that the parasites had been introduced into Herefordshire by Canadian store cattle. There does not appear to be any definite evidence for this and in view of the specificity of the miracidia for particular snail hosts which several workers have reported it seems improbable that the trematode would have been able to establish itself. During the autumn of 1948 the abattoirs at Hereford and Leominster were visited but although many hundreds of sheep and cattle from the surrounding districts were examined no paramphistomes were found.

The first specimens of paramphistomes from this country which were sent to the author were collected from a cow in the Isle of Mull. During a subsequent visit to the island only two beasts out of the two herds whose faeces were examined proved to be infected and the faeces of twenty sheep which had been grazed over the same area were also examined but all were free of paramphistome eggs. None of the infected beasts had been bred on the island but were bought on the mainland three or four years previously. A very large number of snails of various species were collected but none were infected and it therefore

* Part of a thesis approved by the University of London for the award of the Ph.D. Degree.

seems probable that the right species of snail to act as intermediate host was not present and that the parasite had been unable to establish itself.

Two visits were paid to the Municipal Abattoir in Glasgow. On the first occasion three rumens from Irish cattle were found to be infected and on the second out of some five hundred rumens of Scottish cattle examined only one was found to be infected. All the infections were heavy. A third collection, believed to be from Scottish cattle, and three collections from Ireland were sent to the author. It has also been possible to examine two collections from the Netherlands and two from France.

While comparing sections of specimens from the various sources it became obvious that three different species were present. One of these can be identified according to Näsmark's system of classification as *P. cervi* but the other two both show a number of characters which are different from those of any hitherto described species. They are therefore regarded as new, and named and described below.

A very short account of gametogenesis and the early development of both species is given.

Material and Methods.

The material collected from the abattoir, Glasgow, was fixed in 10% formalin, Bouin's fluid or Carnoy (6 : 3 : 1). The specimens from the Isle of Mull had been fixed in 10% formalin, those from Ireland in formol saline, 5% formalin or 70% alcohol ; those from the Netherlands in 70% alcohol or 10% formalin and those from France in 10% formalin. Specimens for sectioning were cleared in cedarwood oil and embedded in paraffin wax with ceresin, congealing point about 54°C. Transverse, horizontal and sagittal sections were cut, the thickness varying from 4μ–10μ. Those 6μ–8μ in thickness proved most satisfactory. Thick hand sections were also cut and a number of worms dissected under the binocular microscope in order to show the appearance of the testes. Serial sections were stained with Ehrlich's haematoxylin and eosin, Weigert's iron haematoxylin without counterstain or counterstained with van Gieson's picrosaurefuchsin, and Heidenhain's iron haematoxylin without counterstain. Hand sections were stained with borax-carmine.

Drawings were made with the aid of a camera lucida. The sagittal sections of the two new species are composite drawings. Measurements were taken on whole worms, and on sections.

Type material of the two new species is deposited in the collection

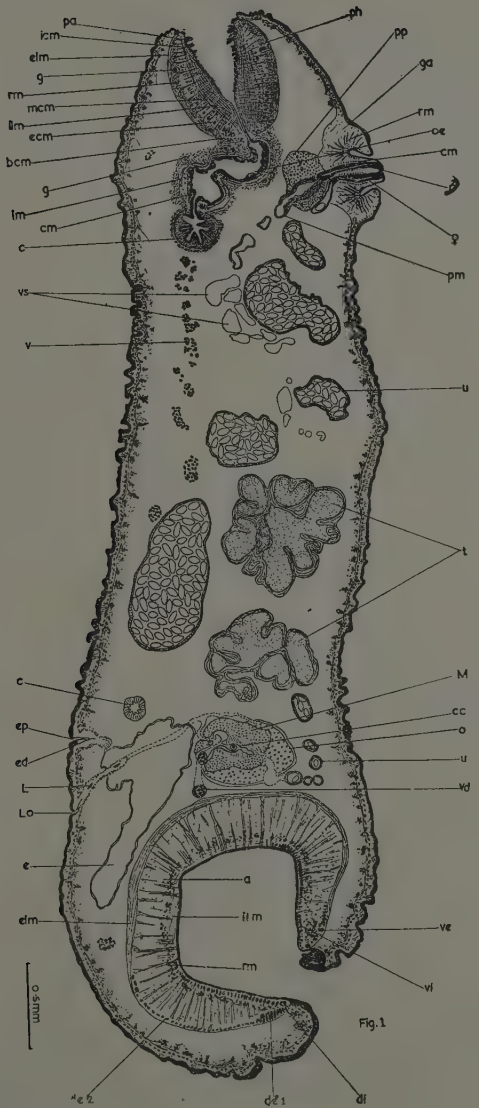


Fig. 1. *Paramphistomum hiberniae* n.sp. Sagittal section.

of the Department of Parasitology of the London School of Hygiene and Tropical Medicine.

Paramphistomum hiberniae n.sp.

Geographical distribution: Ireland, Scotland, The Netherlands.
Host: Bos taurus.
Habitat: Rumen.
Specific diagnosis: Length 4.9 mm., breadth 1.9 mm., dorsal-ventral 1.9 mm. Dorsal line, very slightly curved. Acetabulum, internal diameter, 0.95 mm.; proportion to body length, 1 : 5.7; type, *Paramphistomum.* Pharynx, length 0.71 mm.; proportion to body length, 1 : 7.7; type, modified *Liorchis.* Oesophagus length 0.49 mm. Genital atrium, type, *Ichikawai*, on a level with the oesophagus. Testes, one behind the other, small, almost spherical, very deeply lobed with a muscular sheath. Ovary, ovoid to spherical, posterior to testes. Excretory duct short.

Morphology of *P. hiberniae* n.sp.

Habit: Before fixation the worms were pinkish to red. They were found in large numbers at the bottom of the oesophageal groove and between the villi of the rumen. The body is straight or with an even slight curve.

Dimensions: Measurements taken after fixation.

Length : 4 mm.—7 mm.	Average 4.9 mm.
Breadth : 1.5 mm.—2.2 mm.	Average 1.9 mm.
Dorsal-Ventral : 1.6 mm.—2.2 mm.	Average 1.9 mm.

Abbreviations used in Figs. 1–10.

a—acetabulum; **bcm**—basal circular muscle; **c**—gut caecum; **cc**—central chamber of Mehlis gland; **cm**—circular muscle; **cu**—cuticle; **de 1**—dorsal external circular muscle, first series; **de 2**—dorsal external circular muscle, second series; **di**—dorsal internal circular muscle; **e**—excretory bladder; **ecm**—external circular muscle; **ed**—excretory duct; **elm**—external longitudinal muscle; **ep**—excretory pore; **ep'**—epithelial cell of testis wall; **f**—fertilisation membrane; **fi**—fibrous tissue of testis wall; **g**—gland cell; **ga**—genital atrium; **i**—intracellular ducts; **icm**—internal circular muscle; **ilm**—internal longitudinal muscle; **k**—karyosome; **L**—Laurer's canal; **Lo**—opening of Laurer's canal to exterior; **lm**—longitudinal muscle; **m**—muscle fibre; **M**—Mehlis gland; **Mc**—cells of Mehlis gland; **mcm**—middle circular muscle; **n**—nerve; **nf**—non-fibrous layer of testis wall; **o**—ovary; **oe**—oesophagus; **on**—oocyte nucleus; **pa**—papilla; **pa'**—parenchyma; **ph**—pharynx; **pm**—pars musculosa; **pp**—pars prostatica; **r**—reservoir; **rm**—radial muscle; **sp**—spermatozoon; **st**—spermatogonial tissue; **t**—testes; **u**—uterus; **v**—vitellaria; **vd**—vitelline duct; **ve**—ventral external circular muscle; **vs**—vesicula seminalis; **w**—wall of central chamber of Mehlis gland; ♀—female duct in genital atrium; ♂—male duct in genital atrium.

Acetabulum: Paramphistomum type. Measurements were taken on sagittal sections. The external diameter is taken from the membrane which delimits the tissue of the acetabulum from the body parenchyma; the internal diameter is the diameter of the cavity of the acetabulum.

External diameter:	1.5 mm.—1.7 mm.	Average 1.65 mm.
Internal diameter:	0.9 mm.—1.0 mm.	Average 0.95 mm.
Internal diam./body length:	1/5.0—1/6.2.	Average 1/5.7.
Diameter of opening:	0.2 mm.—0.5 mm.	Average 0.38 mm.

Circular muscles	No. of units	Average
Dorsal external 1	15–23	19
Dorsal external 2	26–39	30
Dorsal internal	42–48	46
Ventral internal	47–58	50
Ventral external	17–22	19

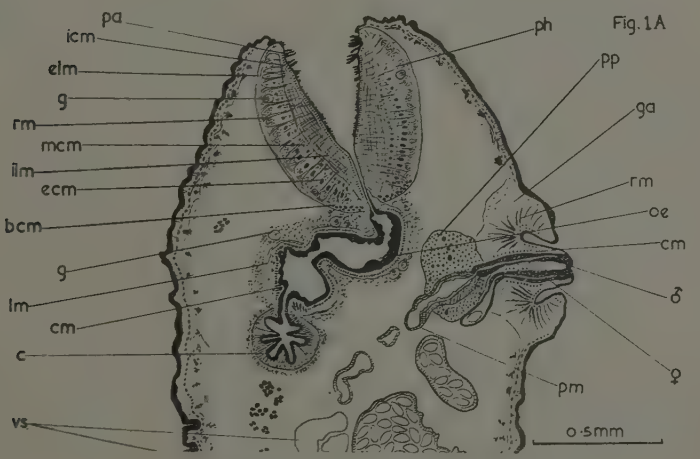


Fig. 1a. *P. hiberniae* n.sp. Genital atrium and pharynx.

Pharynx: (Fig. 1a). Modified *Liorchis* type. The middle and external circular muscle layers are better developed in the posterior two-thirds of the pharynx. At the anterior end they are quite indistinct. The papillae are fairly long round the opening of the pharynx to the exterior but become progressively smaller towards the oesophageal end, where they are inconspicuous or lacking. Under an oil immersion objective it is possible to distinguish strands running into the papillae

from amongst the band of longitudinal and radial muscles. These are believed to be nerves and the papillae to have a sensory function (Fig. 4).* There are also a number of large uninucleate cells with clear cytoplasm which are believed to have a glandular function. Similar cells can be seen in the epithelium surrounding the oesophagus.

Length of Pharynx : 0.64 mm.—0.8 mm. Average 0.71 mm.
Length/Body length : 1/5.4—1/8.8. Average 1/7.7.

Oesophagus : This is fairly straight, short.

Length : 0.45 mm.—0.54 mm. Average 0.49 mm.

Genital atrium: (Fig. 1a). *Ichikawai* type, with very strongly marked radial muscles. The atrium does not lie very far anteriorly and is situated about the level of the middle of the oesophagus.

Testes : (Figs. 1, 2, 3). These are small and spherical and lie one behind the other. Both are about the same size and they are usually some distance apart. The average measurements are : the anterior testis, 0.62 mm. × 0.59 mm. × 0.60 mm. and the posterior, 0.60 mm. × 0.57 mm. × 0.62 mm. They are extremely deeply lobed, as seen both in longitudinal and transverse section and have a very fibrous wall in which both circular and longitudinal muscle elements are present (Figs. 2 and 3).

The spermatogonial cells are small and only comparatively few spermatozoa are produced at a time. This is shown by the very few spermatozoa which are present in the vesicula seminalis although they are often numerous in the uterus. The peculiar development of the testis cannot be due to immaturity as in some specimens the uteri are packed with eggs.

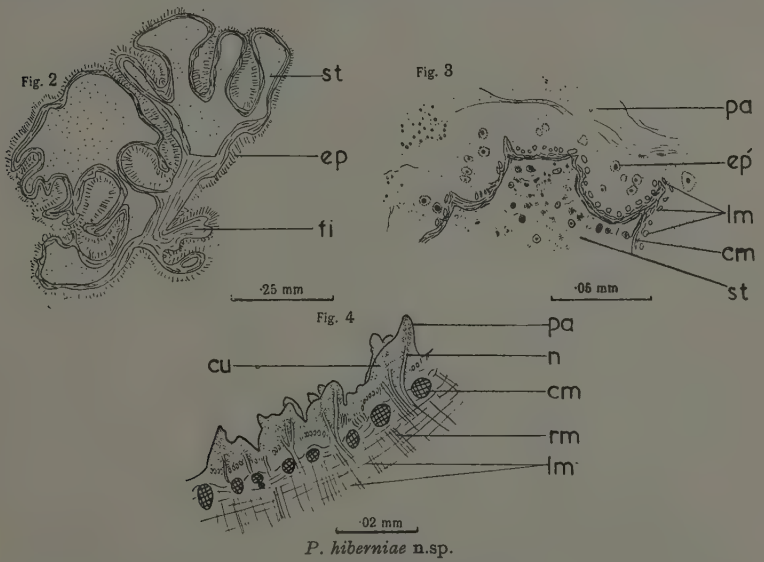
Ovary : (Fig. 1). The ovary lies between the posterior testis and the acetabulum. It is roughly spherical and contains oogonia and primary oocytes. The oviduct leaves the ovary on the anterior border and runs dorsally and slightly posteriorly round Mehlis gland until it joins Laurer's canal. It then runs into Mehlis gland and is joined by the vitelline duct, forming the central chamber.

Mehlis gland : (Fig. 1). This lies very close to, and almost on the same level as the ovary. There is a distinct region of intracellular ducts, very similar in extent to that described for *Gigantocotyle bathycotyle*. Laurer's canal runs from it and is joined by the oviduct just outside Mehlis gland. It then turns posteriorly and dorsally and opens to the exterior a short distance behind the opening of the excretory pore. Although neither vitelline material nor cells were found in Laurer's

* Looss (1896) has also described and drawn these papillae in *Gastrodiscus aegyptiacus* and *Gastrothylax gregarius*. He considered them to be nerve endings.

canal, they were visible in one series in that part of the duct between the central chamber and the junction of the oviduct with Laurer's canal. This indicates that Laurer's canal may serve as a way through which surplus vitelline material may be passed to the exterior as has been suggested.

Uterus: In specimens with only a few eggs the uterus is narrow and only very slightly folded. As it becomes packed with eggs it spreads to occupy almost the whole body between the oesophagus and the testes.



P. hiberniae n.sp.

Fig. 2. Transverse section of testis. Fig. 3. Higher magnification of testis wall. Fig. 4. Pharyngeal papillae.

Vitellaria: These are follicular and extend from the level of the pharynx almost to the posterior end of the worm. There are a pair of vitelline ducts which join between Mehlis gland and the acetabulum to form a small vitelline reservoir. From this a short duct runs into the central chamber of Mehlis gland.

Excretory bladder: (Fig. 1). This is quite large and lies dorsally to the acetabulum but does not extend far anteriorly. It opens to the exterior by a very short duct, the pore being about the same level as the ovary.

Gametogenesis in *P. hiberniae.*

Spermatogenesis: Owing to the small size of the spermatogonial cells and the slowness with which divisions take place spermatogenesis is difficult to follow. As far as it is possible to say at present it proceeds in an exactly similar manner to that described for *Gigantocotyle bathycotyle* (Willmott, 1950). A rosette is formed in which there are thirty-two spermatid nuclei, indicating that there are the usual three spermatogonial divisions followed by two reduction divisions. Very few nuclei could be picked out in stages of mitoses or meiosis and it is impossible to state with certainty how many chromosomes there are. In those cells where nuclear divisions could be seen, normal spindles are formed and the chromosomes become orientated upon them at metaphase. (Fig. 5a.) No centrosomes have been seen. The haploid number of chromosomes is not less than six and not more than eight, eight occurring most frequently.

Oogenesis: Only oogonial divisions take place in the ovary. The oogonia form a cap on the outside of the ovary and the primary oocytes are in the centre and towards the opening of the oviduct. The primary oocytes enter the oviduct and are penetrated by a spermatozoon. The fertilisation membrane has formed by the time the oocyte reaches the central chamber of the Mehlis gland (Fig. 5). The maturation divisions and the formation of the male and female pronuclei have not been observed.

Cleavage: The first cleavage division results in the formation of two unequal cells as has been described for a number of trematodes by various authors. These continue to divide until a vitelline membrane and an embryo of about eight to ten cells has developed. By this time the eggs are in the most anterior part of the uterus and are about to be oviposited. The vitelline membrane is probably derived from the ectodermal or larger cell of the first division, but as in *Gigantocotyle bathycotyle* the brittleness of the egg shell causes a great deal of tearing in the sections and it is very difficult to follow the details of the divisions.

Paramphistomum scotiae n.sp.

Geographical distribution: Scotland, Ireland.

Host: Bos taurus.

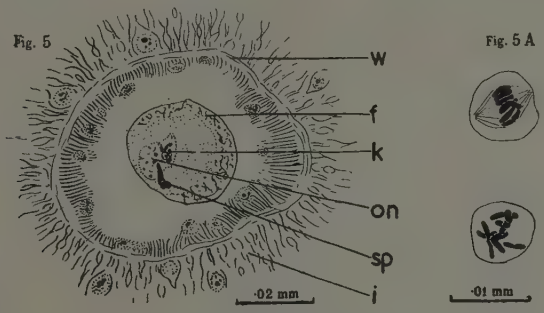
Habitat: Rumen.

Specific diagnosis: Length 5.1 mm., breadth 2.6 mm., dorsal ventral 2.0 mm. Dorsal line strongly curved. Acetabulum, internal diameter 0.8 mm. ; proportion to body length, 1 : 6.4 ; type *Paramphistomum.* Pharynx, length 0.62 mm., proportion to body length

1 : 8.2 ; type, modified *Liorchis* with well-developed papillae. Oesophagus, length 0.6 mm. Genital atrium, type *Epiclitum*, on a level with the posterior part of the oesophagus. Testes, one behind the other, large with few lobes, the posterior often sickle-shaped in sagittal section, without a muscular sheath. Ovary, ovoid to spherical, posterior to testis. Excretory duct short.

Morphology of *P. scotiae* n.sp.

Habit : These paramphistomes were not seen before fixation but they were reported to have been whitish. The body is strongly curved.



P. hiberniae n.sp.

Fig. 5. Primary oocyte in central chamber of Mehlis gland. Fig. 5a. First metaphase chromosomes in primary spermatocytes.

Dimensions :

Length :	3.8 mm.— 6.0 mm.	Average 5.1 mm.
Breadth :	2.0 mm.— 3.0 mm.	Average 2.6 mm.
Dorsal-Ventral:	0.9 mm.— 2.4 mm.	Average 2.0 mm.

Acetabulum : Paramphistomum type. Measurements were taken on sagittal sections :

External diameter :	1.0 mm.—2.0 mm.	Average 1.6 mm.
Internal diameter :	0.4 mm.—1.0 mm.	Average 0.8 mm.
Internal diam./body length :	1/12.7—1/5.1.	Average 1/6.5
Diameter of opening :	0.2 mm.—0.7 mm.	Average 0.4 mm.

Circular muscles	No of units	Average
Dorsal external 1	13–21	18
Dorsal external 2	18–39	27
Dorsal internal	34–45	38
Ventral internal	36–43	38
Ventral external	13–16	15

Pharynx: (Fig. 6a). Modified *Liorchis* type. The pharynx is considerably retracted in all the specimens sectioned forming a funnel above the actual opening. The development of the muscular layers is very similar to that in *P. hiberniae* although the pharynx is shorter and more rounded than in this species. The papillae are more extensive than in *P. hiberniae* and seem to branch in many cases. Gland cells are present.

Length of pharynx : 0.5 mm.— 0.72 mm. Average 0.62 mm.
Length/Body length: 1/10—1/7. Average 1/8.2

Oesophagus: (Fig. 6). This is slightly longer than in the previously described species and not so straight.

Length : 0.8 mm.— 0.9 mm. Average 0.56 mm.

Genital atrium: (Fig. 6a). *Epiclitum* type with fairly strongly developed radial muscles. It does not lie very far forward, being on a level with the posterior part of the oesophagus and the junction of the gut caeca.

Testes: (Figs. 6 and 8). These lie one behind the other and are comparatively large with a few lobes. The anterior one is rounded and the posterior often sickle-shaped in section. They are close together and the average measurements are :—anterior testis, 1.1 mm. × 1.5 mm. × 1.2 mm. ; posterior testes, 1.2 mm. × 1.5 mm. × 1.6 mm. The sheath which surrounds the testis contains no muscular elements (Fig. 8). The spermatogonial cells are much the same in size and appearance as in *Gigantocotyle bathycotyle* and nuclear divisions and spermatozoon formation goes on more rapidly than in *P. hiberniae*.

Ovary: (Fig. 6). The ovary is roughly spherical and lies posterior to the testes and dorsal to the acetabulum. There is a distinct region of oogonia and of primary oocytes. The oviduct leads from the latero-posterior border of the ovary to the outside of Mehlis gland, where it is joined by Laurer's canal. A joint duct leads into Mehlis gland.

Mehlis gland: (Figs. 6 and 7). This lies behind and slightly to one side of the ovary. It is pear-shaped or oval. The region of intracellular ducts is narrow and within it are a number of small round cavities which appear to be lined with some cuticular substance. There is no indication that they are large cells as the contents are quite clear and they are devoid of nuclei. It is thought that they may act as reservoirs for the secretion from the cells of Mehlis gland. They are particularly clear in that part surrounding the part of the uterus which lies within the gland.

Uterus: The uterus in all the specimens examined was packed with

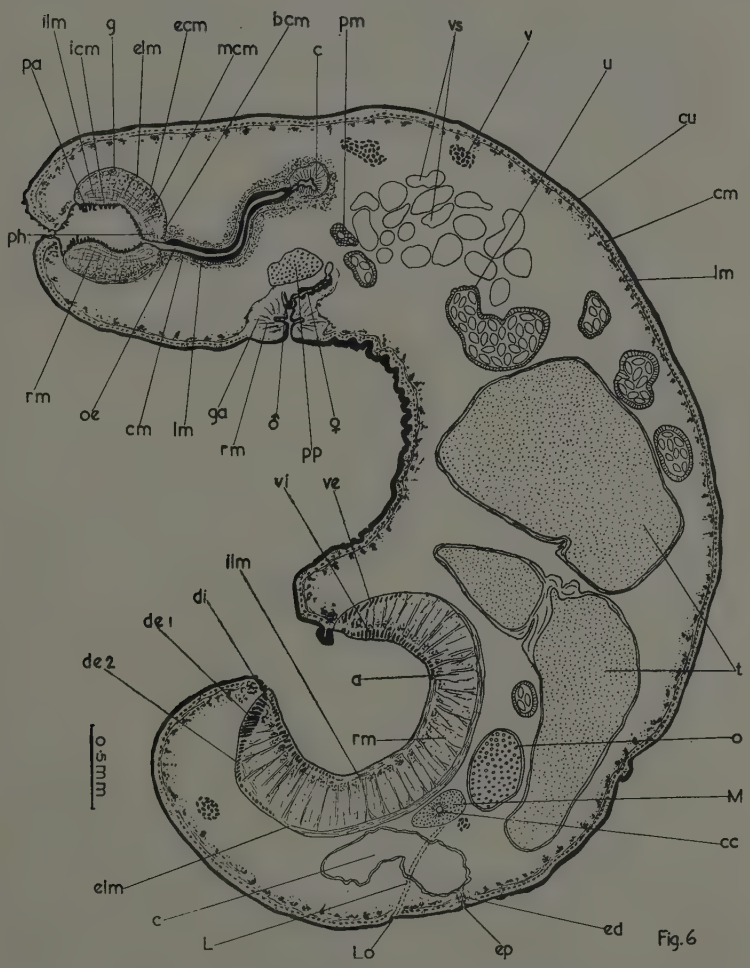


Fig. 6. *Paramphistomum scotiae* n.sp. Sagittal section.

eggs and almost filled the dorsal surface and the space between the testes and the genital atrium.

Vitellaria: These are follicular and extend almost the whole length of the worm. A pair of vitelline ducts runs in and joins forming the

vitelline reservoir, which lies just behind Mehlis gland, and from this a duct runs into the central chamber of the gland.

Excretory bladder: This is large and lies dorsally to the acetabulum. It opens to the exterior by a short duct which runs directly dorsally from the bladder.

Gametogenesis in *P. scotiae.*

Spermatogenesis: The germinal tissue is made up of larger cells than in *P. hiberniae* and it was therefore possible to see most of the stages. The nuclear divisions and spermatozoon formation appear to take place exactly as in *Gigantocotyle bathycotyle.* The chromosome number as seen in meiotic metaphases is, haploid number, eight; diploid, sixteen. Spindles are formed but no centrosomes were seen.

Oogenesis: As before, only oogonia and primary oocytes are found in the ovary, reduction division only taking place after the oocyte has been penetrated by a spermatozoon. This was not observed in this species.

Cleavage: This appears to follow the same plan as that previously described in *G. bathycotyle* and *P. hiberniae.* A vitelline membrane is formed and the embryo is at about the eight to ten cell stage when the egg is laid. Even greater difficulty than before was experienced in getting good sections of the anterior end as the egg shells seem particularly hard and tore the sections badly.

Material from the Netherlands and France.

Two collections from the Netherlands have been received; both were labelled *Paramphistomum cervi* and neither was in a very good state of preservation. One collection had been fixed in 70% alcohol and the other in 10% formalin but in neither could any details of histology or cytology be made out. The acetabulum is the *Paramphistomum* type, the pharynx, the *Liorchis* type and the genital atrium the *Ichikawai* type. These characters, combined with deeply lobed testes indicate that this material is identical with *P. hiberniae.*

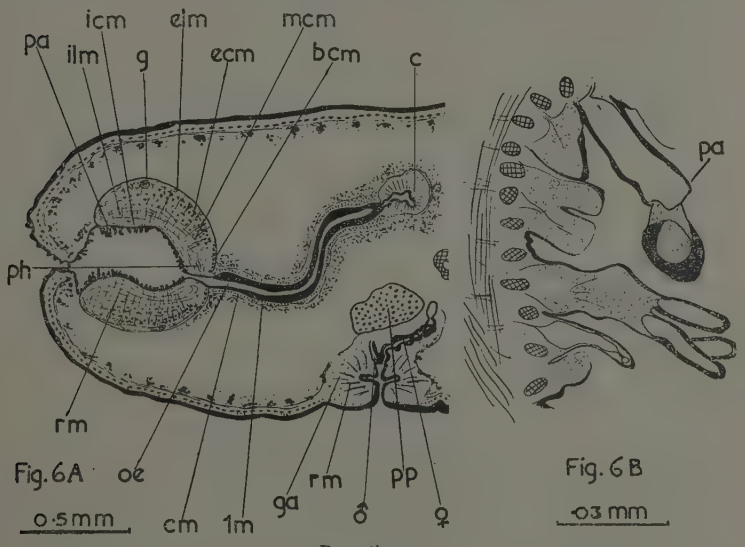
The three specimens labelled *P. cervi* from France are better preserved. There has not, however, been time to make a detailed study of them. From preliminary observations they are very much larger than any of the specimens of *P. scotiae* and *P. hiberniae,* being 8-9 mm. long, 2.5-4 mm. in breadth and 2-2.5 mm. in dorsal-ventral measurement. These agree more closely with* *P. microbothrium* than *P. cervi* according to Näsmark's classification.

* This is the first record of *P. microbothrium* in Europe.

Three specimens identified as *Cotylophoron cotylophorum* have also been received from France. The pathology of this species, and of *P. cervi* has been studied by Guilhon and Priouzeau (1945) who state that *C. cotylophorum* has only once been found in France and that from the district of Meurthe-et-Moselle, in the South East. The papillae in the pharynx of this species are small and inconspicuous.

COMPARISON OF THE NEW SPECIES WITH *P. CERVI* and *P. LEYDENI*.

As has already been stated it was assumed at the beginning of this work that *P. cervi* was the only species present.. There proved, however, to be so many points of difference which were constant between the



P. scotiae n.sp.

Fig. 6a. Genital atrium and pharynx. Fig. 6b. Pharyngeal papillae.

specimens from the different collections that it could not be just a question of individual variation. Unfortunately the two collections which contained *P. cervi* were not fixed by the author and are in rather a poor state of preservation. The genital atrium of these specimens does in some sections show occasional strands of muscle fibres, but these are so irregular and insignificant that the atrium may still be classified as the *Gracile* type. (Fig. 10.)

If the specific diagnosis is based on the acetabulum, pharynx and genital atrium type only, *P. scotiae* might be considered as a synonym of *P. leydeni* Näsmark, 1937, but there are a number of other morphological differences which seem to be sufficient to warrant its description as a new species.

A comparison of the four species *P. cervi*, *P. leydeni*, *P. scotiae* and *P. hiberniae* is given in tabular form in Table I. The figures for *P. cervi* and *P. leydeni* are after Näsmark.

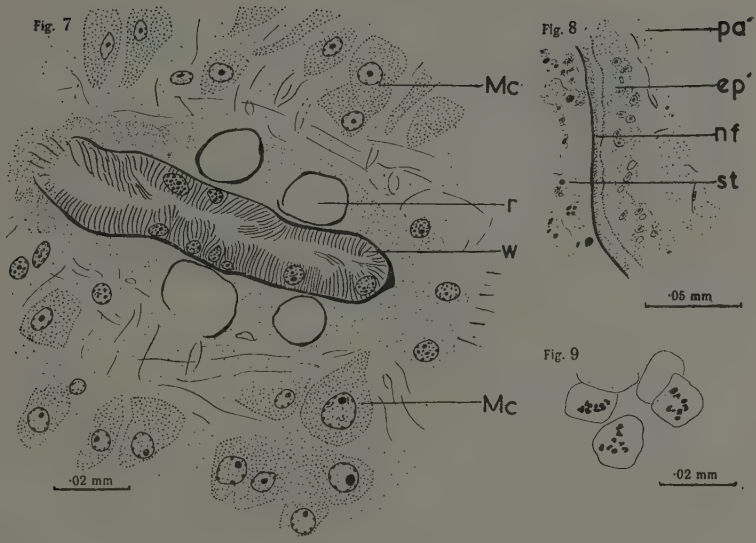
TABLE I.

		P. cervi	*P. hiberniae*	*P. leydeni*	*P. scotiae*
1.	Length	8.88 mm.	4.9 mm.	6.4 mm.	5.1 mm.
2.	Acetabulum diameter/ Body length	1/4.4	1/5.7	1/3.6	1/6.4
3.	Pharyngeal papillae	Small, inconspicuous	Well developed anteriorly	Well developed	Very well developed
4.	Genital atrium	*Gracile* type	*Ichikawai* type	*Epiclitum* type	*Epiclitum* type
5.	Position of genital atrium	Level with posterior part of oesophagus	Level with oesophagus	Level with most anterior part of pharynx	Level with posterior part of oesophagus
6.	Testes	Large, slightly lobed	Small, deeply lobed	Often disc-shaped	Large, slightly lobed
7.	Excretory bladder	Dorsal to acetabulum	Dorsal to acetabulum	Dorsal and anterior to ovary and acetabulum	Dorsal to acetabulum
8.	Excretory duct	Fairly long	Very short	Very long	Very short
9.	Level of excretory pore	Front of posterior testis	Middle of posterior testis	Anterior border of anterior testis	Posterior border of posterior testis

Although not a great deal of importance is attached to the number of circular muscle units in the acetabulum it is interesting to compare the average numbers of *P. hiberniae* and *P. scotiae* with those given by Näsmark for *P. cervi*. He does not give figures for *P. leydeni*.

TABLE II.

Circular muscle units	*P. cervi*	*P. hiberniae*	*P. scotiae*
Dorsal external 1 ..	14	19	18
Dorsal external 2 ..	37	30	27
Dorsal internal ..	41	46	38
Ventral internal ..	40	50	38
Ventral external ..	19	19	15



P. scotiae n.sp.

Fig. 7. Uterus in Mehlis gland. **Fig. 8.** Testis wall. **Fig. 9.** First metaphase chromosomes in primary spermatocytes.

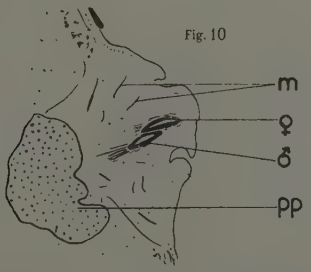


Fig. 10. *P. cervi.* Genital atrium.

Summary.

1. Two new species of *Paramphistomum* from cattle are described, namely, *P. hiberniae* n.sp. and *P. scotiae* n.sp., first collected from Irish and Scottish cattle respectively.

2. A brief account of gametogenesis and early cleavage division is given.

3. The chromosome number for *P. scotiae* is n=8, 2n=16, for *P. hiberniae* no definite number is given but it is believed to be n=8 (6–8), 2n=16 (12–16). Notes are made on some specimens received from the Netherlands and France.

4. *P. hiberniae* and *P. scotiae* are compared with *P. cervi* and *P. leydeni*.

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The author wishes to express her appreciation of the following who have so kindly given her material:

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Finally she wishes to thank Professor J. J. C. Buckley who has supervised the work, and Mr. F. R. N. Pester, without whose help in the Isle of Mull, Glasgow and London the work could not have been carried out.

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*On *Enterobius vermicularis* (Linnaeus, 1758) and Some Related Species from Primates and Rodents.

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In the course of an investigation into the parasitic helminths of Malaya, *Enterobius vermicularis* from man was examined critically. It was considered desirable to compare it with *E. vermicularis* collected in London and with species of *Enterobius* from Primates and Rodents.

E. vermicularis (Linnaeus, 1758) in Man.

Hsü (1933) points out that although Leuckart (1876) recorded six pairs of caudal papillae, the small pair of sessile papillae (Fig. IA) situated lateral to the most anterior pair has been overlooked by modern investigators. Baylis (1936) mentions only five pairs of caudal papillae in *E. vermicularis*. To verify this point about twenty males obtained both from London and Malaya were examined. Examining in the ordinary way under the cover-glass the small papillae were seen only in thee of the specimens. However, when the cut tail end was mounted in the clearing medium as a hanging-drop preparation (a very useful technique in use by Professor Buckley for the examination of the *en-face* view of the head, ventral view of the tail, etc.) the small sessile papillae could be easily seen in the vast majority of the specimens both from Malaya and London. This technique permits of examination in any of the clearing media and the specimen can be adjusted to any required position. This particular papilla is so situated as to be easily pressed upon by the cover-glass and is not readily seen from all angles and probably accounts for the difficulty experienced in seeing it.

There seems to be some doubt as to the status of the broader basal portion of the spicule. In giving the measurement of the spicule as being 70μ in length, many authorities such as Brumpt (1949), Faust (1949), Blacklock and Southwell (1940) and Belding (1942) do not include the basal part. Baylis (1936) and Pinto (1938) include the basal portion and give the length of the spicule as being 120–130μ.

* Part of a thesis approved by the University of London for the award of the Ph.D. Degree.

A careful examination of many males from Malaya and London shows that the basal portion varies considerably both in the degree of refractility and in its length. Whereas the length of the distal tubular portion is remarkably constant in the whole series varying only from 0.072 to 0.078 mm., the basal portion varies from 0.028 to 0.054 mm. Nevertheless, the basal portion should be considered as a part of the spicule but owing to its variable nature, measurements of it should be given separately.

Examination of Spicules of *Enterobius* spp. with Polarized Light.

At Professor Buckley's suggestion, the spicules were examined with polarized light in specimens of *E. vermicularis* which had been cleared in media of varying refractive indices namely creosote, glycerine or lactophenol. In all these, it was found that the walls of the distal tubular portion were birefringent whereas the basal portion was isotropic in polarized light. The examination of spicules in the various media of different refractive indices shows no appreciable difference in their birefringence suggesting that this was due to the intrinsic properties of the substances comprising the spicule (Pantin, 1948). The spicules of *E. anthropopitheci* and *E. buckleyi* (described below) were similarly examined with polarized light and again the basal portion was found to have no birefringent properties. The results of the examination of the spicules of *Enterobius* spp. by the use of polarized light shows some similarity to the findings of Crusz (1948) with the rostellar hooks of taeniids. Although in these hooks the base is firmly associated with the blade to form a rigid structure, he finds that the blade is birefringent while the base, at all stages of its development, is isotropic in polarized light. He concludes that there exists a structural difference between the proteins of the base and blade which is correlated with the fact that while the blade is remarkably constant in shape and proportions within a species, the base is more variable and is often the seat of most of the anomalies in hook structure.

E. vermicularis in a Chimpanzee.

The material consists of one male and six females collected by Dr. R. E. Rewell from a young chimpanzee in the London Zoological Gardens. Peri-anal (NIH) swabs taken from seven chimpanzees in the London Zoological Gardens on 20th November, 1949, showed one positive. The eggs measure 74μ by 37μ. In the adult worms the cuticular striations are prominent and lateral alae are present. There are three lips which project about 11μ in front of the cuticular expansion.

The spicule (Fig. IB) is relatively stout and has the characteristically recurved blunt tip, like a crochet-hook. Its total length is about 0.097 mm., the distal tubular portion being 0.076 mm. long. The basal portion, which embraces the anterior extremity of the more highly chitinized and narrower distal portion, measures 0.03 mm. in length. Only five pairs of caudal papillae could be seen. The taxonomically significant measurements are given in Table I.

E. vermicularis in a Lar Gibbon from Malaya.

Two collections of *Enterobius* from the Malayan Waa-Waa or Lar Gibbon (*Hylobates lar*) which had been in the London Zoological Gardens proved to be *E. vermicularis*. There was only one male but the collection contained several females.

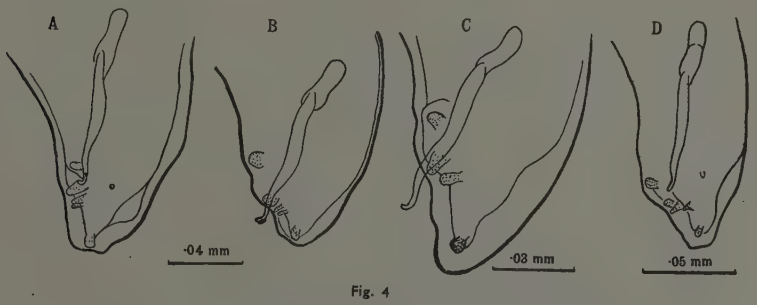

Fig. 4

Fig. 1. *Enterobius vermicularis*, Posterior end of male.
A, from Man ; B, from Chimpanzee ; C, from Lar Gibbon ; D, from Marmoset.

The spicule (Fig. IC) measures 83μ including the basal portion, the distal tubular portion being 70μ. Although only four pairs of caudal papillae could be seen in this slightly damaged specimen, the characteristic shape of the spicule is sufficient to be able to refer it to *E. vermicularis*. The important measurements are given in Table I.

E. vermicularis in the Lion Marmoset.

The material consists of a single male and several females collected at the London Zoological Gardens from the Lion Marmoset (*Leontocebus rosalia*) from South East Brazil.

In this male specimen, the distal tubular portion of the spicule (Fig. ID) is slightly shorter and the curvature at the tip is much less pronounced than in the typical *E. vermicularis*. The important

measurements in millimetres are given in Table I together with those of *E. vermicularis* from the Lion Marmoset and Lar Gibbon for comparison.

Discussion on *E. vermicularis* in Primates.

Stiles and Hassall (1929) record *E. vermicularis* from *Pan* species but both Professor R. T. Leiper of the Commonwealth Bureau of Agricultural Parasitology and Dr. E. W. Price of the U.S. Department of Agriculture have been unable to trace the original source of this report.

Hamerton (1942) reports *E. vermicularis* from *Gorilla gorilla* but it seems very probable that it is the same material forwarded to Professor Buckley by Dr. Annie Porter and described as a new species herein.

A Description of the hitherto unknown Male of *E. anthropopitheci* (Gedoelst, 1916) from a Chimpanzee and a Re-description of the Female.

Gedoelst (1916) described *E. anthropopitheci* from the female specimens only. Baylis and Daubney (1922) obtained some specimens from a black-headed lemur in India which they tentatively described as *E. anthropopitheci*. Their material also consisted only of females. Pillers (1921) records obtaining on post-mortem examination 50–60 "*Oxyuris* sp." in the large intestine of a two-year old chimpanzee imported to Liverpool from West Africa. Stiles and Hassall (1929) record *E. vermicularis* from *Pan* sp. (see comments above.) Moorman (1941) records finding one female pin-worm in a chimpanzee in U.S.A. but there appears to have been no attempt to determine the species. Porter (1946) records finding an unnamed species of *Enterobius* in a chimpanzee in the London Zoological Gardens.

Numerous male and female pin-worms were collected by Dr. P. L. LeRoux from a chimpanzee (*Anthropopithecus troglodytes*) which died in this laboratory soon after its arrival in London. The description which follows is based on this material.

Morphology (Fig. 2).

The transverse striations of the cuticle are prominent and its anterior end is inflated. The cuticular inflation appears globular in young specimens but in most of the older forms, especially the females, the anterior portion is invaginated withdrawing the lips into the depression thus formed. Prominent lateral ridges run through the whole length of the body in both sexes.

Female: The gravid specimens measure 4.9 to 5.6 mm. in length

TABLE I.—Measurements of *E. vermicularis* from Primate hosts.

Host	Chimpanzee		Lar Gibbon		Lion Marmoset	
Sex	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*
Length	2.5	6.52–7.2	2.3	4.6–5.1	3.5	7.6–8.15
Maximum width	0.18	0.4	0.15	0.33–0.4	0.16	0.37–0.48
Length, cut. inflation	0.18	0.19–0.22	0.13	0.12–0.2	0.15	0.23–0.24
Max. width, cut. inflation	0.096	0.15–0.16	0.1	0.13–0.18	0.09	0.15–0.19
Nerve ring from ant. end	0.15	0.2–0.26	0.1	0.17–0.24	0.16	0.19–0.22
,, ,, divides Oesoph.	1 : 3.6	1 : 4–5	1 : 4.9	1 : 2.5–3.4	1 : 2.4	1 : 3–3.7
Length oesophagus	0.54	1.0	0.49	0.73–0.8	0.55	0.86–0.9
,, post. oesoph. bulb	0.11	0.19–0.2	0.08	0.16	0.13	0.17–0.18
Diameter ,, ,, ,,	0.074	0.15–0.16	0.067	0.14–0.15	0.08	0.15–0.16
,, ant. ,, ,,	0.063	0.085–0.09	0.056	0.08	0.048	0.09
,, narrow ant. oesoph.	0.033	0.05–0.056	0.03	0.04–0.056	0.026	0.044–0.048
Vulva to tip of tail		4.6–4.97		3–3.5		5.12–5.8
,, divides body		1 : 2.2–2.4		1 : 1.9–2.2		1 : 2–2.5
Egg		63–65 × 26–28		52–59 × 26–30		63–65 × 25–28
Tail		1.34–1.5		1.14–1.3		1.56–1.58
Spicule, total length	0.097		0.083		0.094	
,, distal portion	0.076		0.07		0.064	
α	14	16.3–18	15.3	12.75–14	22	17–20.5
β	4.6	6.52–7.2	4.7	6.1–6.4	6.4	8.8–9
γ		5		3.8–4		5–5.2

and 0.45 to 0.54 mm. in maximum breadth. The cephalic cuticular inflation measures about 0.13 mm. in length and the maximum diameter varies from 0.11 to 0.12 mm. The oesophagus measures 0.83 to 0.89 mm. in length ; the posterior oesophageal bulb measures 0.11 to 0.13 mm. in length and 0.1 to 0.11 mm. in diameter. The anterior bulb is 0.074 mm. in diameter and the narrow part joining it to the mouth measures 0.044 to 0.05 mm. in diameter. The nerve ring surrounds the oesophagus at a distance of 0.17 to 0.18 mm. from the anterior end, its position dividing the oesophagus in the ratio of 1 : 4. The excretory pore is at the level of the commencement of the intestine. The vulva divides the body in the ratio of 1 : 2–2.6. In young specimens it can be seen that the muscular ovejector is directed posteriorly when it continues as a thin-walled distended portion which meets the two uteri. The uteri then double on themselves and run in opposite directions. The anterior ovarian tubule bends forward when it reaches the level of the vulva, so that the commencement of both ovarian tubules lies in the anterior third of the body. The eggs have a relatively thick-walled shell and measure 56 by 24μ and are slightly flattened on one side. The tail is long and tapering and measures 1.34 to 1.38 mm. in length. α=10.4–10.9 ; β=6–6.3 ; γ=3.6–4.2.

Male : It measures 1.5 to 1.9 mm. in length and 0.12 to 0.16 mm. in maximum breadth. The cephalic cuticular inflation measures 0.067 to 0.078 mm. in length and projects 0.017 to 0.02 mm. on either side of the body, giving it a diameter of 0.066 to 0.078 mm. The oesophagus measures 0.36 to 0.44 mm. in length. The posterior oesophageal bulb is 0.067 to 0.078 mm. long and has a diameter of 0.063 to 0.067 mm. The anterior bulb has a diameter of 0.037 to 0.04 mm. and the narrow part joining it to the mouth is 0.037 mm. wide. The nerve ring surrounds the oesophagus at a distance of 0.11 to 0.13 mm. from the anterior end, its position dividing the oesophagus in the ratio of 1:2.4. The testis reaches up to the level of the commencement of the intestine in some specimens before turning posteriorly.

The tail is directed ventrally and is truncated. The caudal alae are supported by a pair of large papillae anteriorly and by a pair of elongated rays, the apices of which appear branched ending in three small rounded papillae. Between these are four more pairs of papillae, the most anterior of which is large and appears to be adanal in position. The spicule, including the small basal thickening (5μ wide) measures 0.052 to 0.056 mm. in length. It is fairly straight and ends in an oval knob-like thickening at the tip. The shaft of the spicule tends to become broader towards the centre and the outline of the anterior

border is interrupted by a spur-like projection. There appears to be a fine membrane bridging across the gap. There is no accessory piece. α= 11.5 to 13.7 ; β= 3.7 to 4.5

Discussion on *E. anthropopitheci.*

The female conforms very closely to the description of *E. anthropopitheci* as described by Gedoelst (1916) except in the measurements of the egg where there is a marked difference. Fairly large differences in the size of eggs are recorded in *E. vermicularis* various authorities giving the range as 50 to 60μ by 20 to 32μ. Gedoelst has drawn attention to the globular appearance of the cephalic swelling and the tendency

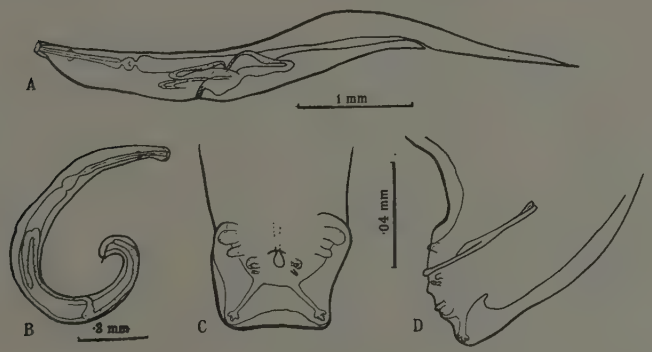

Fig. 2

Fig. 2. *Enterobius anthropopitheci.*

A, lateral view of female ; B, lateral view of male ; C, ventral view of posterior end of male ; D, lateral view of posterior end of male.

for the lips to be withdrawn to the base of a capsule formed by it. He does not include drawings with his description and the female is therefore figured here.

Gedoelst considers *E. microon* (v. Linstow) to be the most closely related species and differentiates it by the shape of the head and size of eggs (42 by 23μ). The great length of the oesophagus in the male *E. microon* and the fact that it is a parasite of a New World Monkey would also serve to exclude this species from our consideration.

There is some resemblance to *E. bipapillatus* (Gedoelst, 1916) in the appearance of the tip of the spicule as illustrated by him although his description reads " une extremité effilée légèrement condée." It

seems probable he was dealing with two different species. In *E. bipapillatus*, however, the spicule is 80μ long with a rounded swollen head 12μ wide and there are only 4 pairs of caudal papillae. Besides, in *E. anthropopitheci* the two prominent lateral cephalic papillae are not present.

Enterobius buckleyi n.sp. from the Orang Utan.

The material here described consists of three males and eight females belonging to the genus *Enterobius* Leach, 1853, obtained from the appendix of an Orang Utan (*Pongo pygmaeus*) which died in the laboratory and kindly placed at the disposal of the writer by Professor Buckley. In addition to the *Enterobius* there was an infection of *Ascaris lumbricoides*, *Trichuris trichiura* and *Dirofilaria* sp. NIH swabs taken from four Orang Utans in the London Zoological Gardens on 20th November, 1949, were all negative.

Two species of *Enterobius* have been described from the Orang Utan, namely *E. faecundus* (v. Linstow, 1879) and *E. simiae* (MacCallum, 1921). From the fact that Cameron (1929) has not provided any different or additional data about these species in his useful review of the species of *Enterobius* in Primates, it seems probable that he had no specimens of these for examination. He expresses the opinion that *E. simiae* is possibly identical with *E. faecundus*. MacCallum's description is totally inadequate according to modern standards. His illustration of the anterior and posterior ends of the female without any accompanying scale adds little information of taxonomic value to his verbal description. His statements that " the vagina is at about the beginning of the posterior third of the worm " and that " both sexes are much alike in appearance " are at complete variance with our knowledge of other members of this genus. *E. simiae* should be treated, therefore, as *nomen inquirendum*. The ensuing description of the present material will show that it differs sufficiently from *E. faecundus* to merit separate specific rank.

Morphology (Fig. 3).

The cuticle is transversely striated and narrow lateral alae are present. The shape of the oesophagus is typically Oxyurid. The anterior end of the oesophagus terminates in three lips, of which the dorsal is the larger. The lips extend 12–15μ anterior to the cuticular inflation. There are no teeth present but the apices of the lips are prolonged forwards and inwards to form small apical processes. There are six cephalic papillae, there being two on each lip. No cervical

papillae are seen. The excretory pore is at the level of the posterior bulb of the oesophagus.

Female: The gravid specimens measure from 6.96 to 7.25 mm. in length and the maximum breadth is 0.37 to 0.42 mm. The vulva is anterior to the middle of the body, and divides it in the ratio of 1 : 1.7 to 1.78. The genital organs are similar to those in *E. vermicularis.*

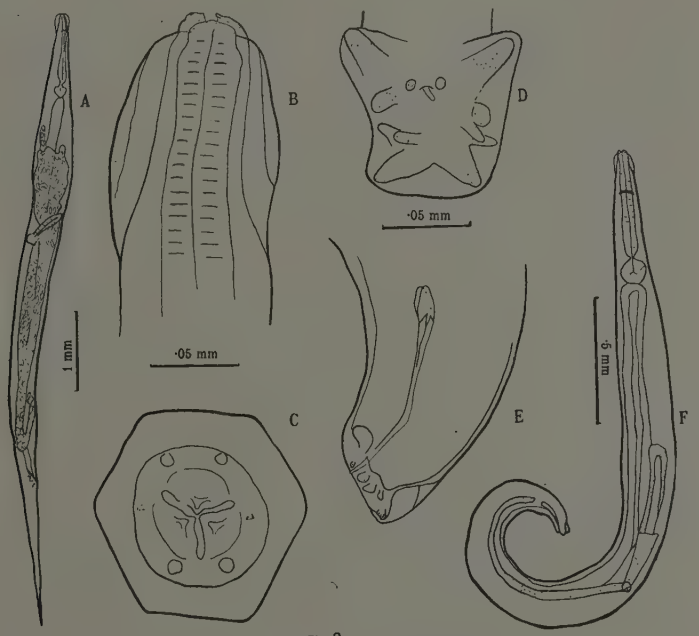

Fig. 3

Fig. 3. *Enterobius buckleyi* n.sp.
A, lateral view, adult female ; B, lateral view, anterior end of female ; C, *en-face* view of head of female ; D, ventral view, posterior end of male ; E, lateral view, posterior end of male ; F, lateral view, adult male.

The eggs are 56μ long by about 26μ broad, somewhat flattened on one side and the shell is smooth.

The cephalic cuticular inflation measures 0.14 to 0.19 mm. in length and projects 0.025 to 0.03 mm. on either side of the body. The oesophagus measures 0.9 to 0.99 mm. in length. The posterior oesophageal bulb varies from 0.17 to 0.185 mm. in diameter, and its length is

similar. The anterior bulb is 0.1 mm. in breadth and the narrow part joining it to the mouth is 0.05 mm. in diameter. The nerve ring surrounds the oesophagus at a distance of 0.17 to 0.18 mm. from the anterior end, its position dividing the oesophagus in the ratio of 1 : 4.3 to 4.5. The excretory pore is at the level of the posterior bulb. The tail is long and tapering and measures 1.35 to 1.43 mm. in length. α=17.3 to 18.8 ; β=7.3–7.7 ; γ=5–5.15.

Male : It measures 3 mm. in length and 0.22 mm. in its maximum width. The cephalic cuticular inflation measures 0.11 mm. in length and projects 0.018 mm. on either side of the body. The oesophagus measures 0.57 mm. in length. The posterior oesphageal bulb is 0.12 mm. long and has a diameter of 0.1 mm. The anterior bulb has a diameter of 0.06 mm. and the narrow part joining it to the mouth is 0.037 mm. broad. The nerve ring surrounds the oesophagus at a distance of 0.15 mm. from the anterior end, its position dividing the oesophagus in the ratio of 1 : 2.8. The testis reaches up to 1.2 mm. from the anterior end before turning posteriorly.

The tail is directed ventrally and is truncated. There are six pairs of caudal papillae of which two are pre-anal in position. The anterior and posterior pairs are particularly large and support a cuticular expansion. The second pair of papillae are smaller than the rest and are situated immediately in front and to the side of the anal opening. The spicule is 0.1225 mm. in length including the broader basal portion which measures about 0.017 mm. in length. The spicule gradually tapers to a rounded tip ; at about two-thirds of its way down it bends forward to form an obtuse angle bridging over which there appears to be a fine membrane. The basal portion of the spicule appears as a double plate the distal part embracing the proximal end of the main part of the spicule. There is no accessory piece. α=13.6 ; β=5.3.

Relationships of *E. buckleyi*.

According to v. Linstow, the mouth of *E. faecundus* shows two lips, and there are no lateral membranes. The oesophagus in the female is 1/6.4 of the body length. The male is 1.8 mm. long and the tail is said to be 1/25 of the body length and to carry a single pair of small post-anal papillae. The spicule is almost straight and measures 0.052 mm. in length. The tail in the female is 1/6 of body length.

The above points in the description do not coincide with the findings in the present species, which differs notably in the presence of six pairs of caudal papillae and a bent spicule which is more than twice as long

as that of *E. faecundus*. A careful examination of the type material may yet show that this form is identical with *E. faecundus* (v. Linstow) but in the absence of such an opportunity, and in view of the marked differences in the descriptions, the writer feels that there is no option but to create a new species.

The species of *Enterobius* here described has been compared with the other species recorded from man, anthropoid apes and the Old-World Monkeys. The straight tip, the shape and length of the spicule serve to distinguish it from *E. vermicularis* and *E. bipapillatus* (Gedoelst, 1916). It differs from the female *E. anthropopitheci* (Gedoelst, 1916) in which the egg is 70 × 32μ and α, β and γ are 11.1 to 12.3, 5.75 and 3.37 respectively. The size and appearance of the spicule (see description above) are also very different in *E. anthropopitheci*.

In *E. pitheci* Cameron, 1929 from *Pithecus aygula* from Assam, only the female is described and β is 10–12 and the eggs measure 50 × 25μ. If, as suggested by Cameron, the specimens from the Indian Monkey *P. entellus* referred by Baylis (1923) to *E. bipapillatus* really refer to *E. pitheci*, then the size of the spicule which is only 60μ long would further serve to exclude *E. pitheci* from our consideration.

This species is named after Professor J. J. C. Buckley.

Family: Oxyuridae Cobbold, 1864.

Species: *Enterobius buckleyi* n.sp.

Host: *Pongo pygmaeus* (Orang Utan).

Location: Appendix.

Locality: Borneo.

Enterobius lerouxi n.sp. from the Gorilla.

The material here described consists of one undamaged and two damaged males and several females obtained after treatment from the faeces of a young Gorilla (*Gorilla gorilla*) in the London Zoological Gardens and sent by Dr. Annie Porter to Professor Buckley. There are no records in literature of an *Enterobius* from the gorilla and this appears to be a new species. NIH swabs taken from a gorilla in the London Zoological Gardens on 20th November, 1949, showed *Enterobius* eggs measuring 67 by 30μ.

Morphology (Fig. 4).

The cuticle is transversely striated and lateral alae are present. The mouth is surrounded by three prominent lips (one dorsal and two sub-ventral). The cuticle is inflated anteriorly and the oesophagus is typically Oxyurid in shape. The lips extend about 15μ in front of the cuticular inflation.

Female: The gravid specimens measure from 5.54 to 6.2 mm. in length and the maximum breadth is 0.26 to 0.29 mm. The vulva is about 2 mm. from the anterior end and its position divides the body in the ratio of 1 : 1.9 to 2.0. The genital organs are similar to those in *E. vermicularis*. The eggs are somewhat flattened on one side, with a smooth thin shell and measure 50 to 63μ long by 26 to 30μ broad.

The cephalic cuticular inflation measures 0.17 to 0.19 mm. in length and the maximum diameter of the inflation is 0.11 mm. The oesophagus is 0.82 to 0.88 mm. long. The posterior oesophageal bulb is 0.15 to 0.16 mm. in length and 0.12 to 0.14 mm. in diameter. The diameter of the anterior oesophageal bulb is 0.07 to 0.08 mm. while that of the narrow part joining it to the mouth is 0.04 mm. The nerve ring surrounds the oesophagus at a distance of 0.19 to 0.2 mm. from the anterior end, its position dividing the oesophagus in the ratio of 1 : 3.3. The tail is 1.3 mm. in length. α= 2.3 to 21.4; β=6.76 to 7; γ=4.3 to 4.8.

Male: It measures 1.92 mm. in length and 0.13 mm. in its maximum breadth. The cephalic cuticular inflation measures 0.12 mm. in length and its maximum diameter is 0.07 mm. The oesophagus measures 0.47 mm. in length. The posterior oesophageal bulb is 0.1 mm. long and has a diameter of 0.074 mm. The anterior bulb has a diameter of 0.04 mm. and the narrow part joining it to the mouth is 0.03 mm. wide. The nerve ring surrounds the oesophagus at a distance of 0.133 mm. from the anterior end, its position dividing the oesophagus in the ratio of 1 : 2.5. The testis reaches up to 0.8 mm. from the anterior end before turning posteriorly.

The truncated posterior extremity is ventrally curved. The caudal alae are supported by four pairs of large pedunculated papillae (one pair pre-anal, one pair adanal and two pairs post-anal). There are in addition three small pairs of papillae (one medial and pre-anal, one between the two post-anal pedunculated pairs and one dorsal to the root of the most posterior pedunculated pair). The spicule is somewhat bent ventrally at about the middle and tapers gradually to a point. The spicule is surrounded by a wide sheath and the whole spicule measures 66μ in length. There is no gubernaculum. α= 15; β=4.1.

Relationships of *E. lerouxi*.

The shape and size of the spicule and the number and position of the caudal papillae in *E. lerouxi* would serve to distinguish it from the known species of *Enterobius*. The male *E. pitheci* is unknown but the female differs in α, β and γ and in the size of eggs. There is some general resemblance to the appearance of the spicule in *E. faecundus*

(described from the Orang Utan from Borneo) as illustrated by Gedoelst but in the latter species the mouth has two lips, there are no lateral membranes, the male tail is 1/25 of body length and there is only a single pair of small post-anal papillae. In *E. faecundus* the

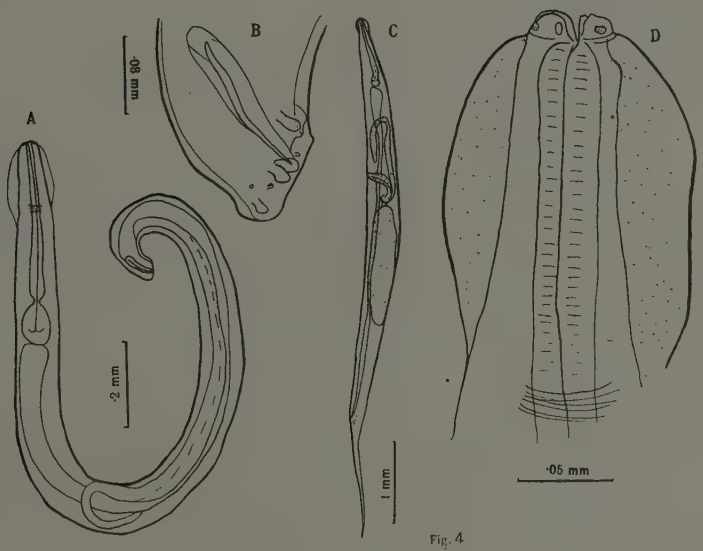

Fig. 4

Fig. 4. *Enterobius lerouxi* n.sp.

A, lateral view of male ; B, lateral view of male tail ; C, lateral view of female ; D, lateral view of head.

spicule measure 52μ and α, β and γ are different. The species is named after Dr. P. L. LeRoux.

Family : Oxyuridae Cobbold, 1864.

Species : Enterobius lerouxi n.sp.

Host : Gorilla gorilla.

Location : In faeces after anthelmintic treatment.

Locality : Recent arrival to London Zoological Gardens from Tropical Africa.

Enterobius bipapillatus (Gedoelst, 1916), in the Guenon Monkey.

The material consists of several males and females collected by Dr. W. K. Blackie from the Guenon Monkey (*Cercopithecus aethiops*) in Southern Rhodesia.

E. bipapillatus was first described by Gedoelst (1916) from an undetermined monkey in Central Africa. It seems probable that Gedoelst was dealing with two species when he described *E. bipapillatus* because in the illustration, the tip of the spicule appears to be rounded whereas according to his description the spicule has a finely drawn out tip which is slightly bent. Baylis (1923) found pinworms in the Indian Langur (*Pithecus entellus*) which he tentatively determined as *E. bipapillatus*. Cameron (1929), however, feels that these should be referred to *E. pitheci* which he described from the Mitred or Capped Monkey (*Pithecus aygula*) in Assam. Cameron (1929) records *E. bipapillatus* from the Green Monkey (*Cercopithecus sabaeus*) from West Africa. Unfortunately his male specimen was poorly preserved and he could not describe the spicule or be sure of the caudal papillae.

Scheidegger and Kreis (1934) redescribe *Oxyuris bipapillatus* Gedoelst, 1916, from the chimpanzee (*Anthropopithecus troglodytes*). They consider it as probably being synonymous with *O. faecundus* v. Linstow, 1879, obtained from the Orang Utan (*Pongo pygmaeus*) in Borneo. Their specimen does not show two cephalic papillae on each lip with the prominent lateral papillae (from which its name is derived) on the ventral lips. According to Gedoelst, the spicule in *E. bipapillatus* is 80μ long with a fine tip whereas in the specimens of these authors it is 51–57μ (av. 54μ) in length and has a rounded tip. It is difficult to agree with their retention of this primate parasite in the genus *Oxyuris* Rudolphi, 1809, in which there are no cephalic cuticular expansions but in which a vestibule with a complicated chitinous armature exists. While it is not possible to form a definite opinion on their specimens without examining them, it would seem possible that they were dealing with *E. anthropopitheci* (Gedoelst, 1916) which is redescribed above.

Morphology (Fig. 5).

There are three prominent lips which project slightly beyond the cuticular inflation. There are six cephalic papillae of which the lateral ones on the ventral lips are large and finger-like. The transverse cuticular striations are distinct.

The gravid female measures about 4.8 mm. in length. The vulva is at the junction of the anterior and middle thirds and the egg measures 61–65μ long by 28–29μ broad. The nerve ring divides the oesophagus

in the ratio of 1 : 2.8–3.3. α=13.7–14 ; β=6.84–7 ; γ=4.17–4.35.

The male measures about 2 mm. in length. The tail is truncated and curved ventrally. The spicule measures 76–85μ in length and has a base which is about 8μ across. There are five pairs of caudal papillae. The nerve ring divides the oesophagus in the ratio of 1 : 1.8–2.2 α=11.7 ; β=4.7–7.

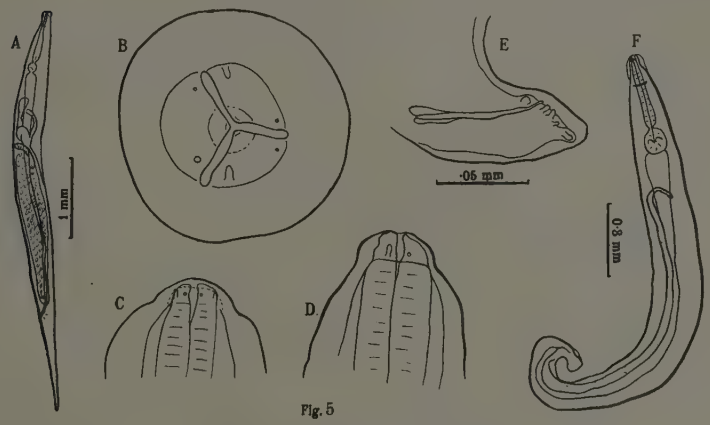

Fig. 5

Fig. 5. *Enterobius bipapillatus.*

A, lateral view of female ; B, *en-face* view of head ; C, ventral view of head ; D, lateral view of head ; E, lateral view of posterior end of male ; F, lateral view of male.

Other measurements of importance are given below (in millimetres):

	Male.	*Female.*
Total length	1.99–2.1	4.79–4.8
Maximum width	0.17–0.18	0.34–0.35
Length of cuticular expansion	0.085–0.09	0.09
Maximum diameter of cut. expansion ..	0.08	0.11
Oesophagus, total length	0.31–0.42	0.7
Posterior oesophageal bulb, length	0.1–0.11	0.15
„ „ „ diameter ..	0.08	0.137–0.14
Anterior oesophageal bulb diameter ..	0.06	0.09
Narrow part of oesophagus, diameter ..	0.03	0.048–0.05
Length of tail	—	1.1–1.15
Vulva to posterior extremity	—	3.18–3.26

Enterobius brevicauda n.sp. from the Chacma Baboon.

The material here described consists of three males and several females obtained from the large intestine of the Chacma Baboon (*Papio porcarius*) from Southern Rhodesia.

Morphology (Fig. 6).

This species resembles *E. bipapillatus* very closely. The cuticle is transversely striated and narrow lateral alae are present. There are three lips and the cephalic papillae are as in *E. bipapillatus*.

Female: They measure 5.38–5.7 mm. in length and the maximum breadth is 0.5–0.6 mm. The vulva is anterior to the middle of the body and divides it in the ratio of 1 : 1.9–2.3. The genital organs are similar to those in *E. vermicularis*. The eggs are 59μ by 26–28μ and somewhat flattened on one side.

The cephalic cuticular inflation measures 0.15 mm. in length and the maximum diameter at the level of inflation is 0.17 mm. The oesophagus measures 0.68–0.77 mm. in total length. The posterior oesophageal bulb measures 0.15 to 0.18 mm. in length and 0.13 to 0.155 mm. in diameter. The anterior bulb is 0.08 to 0.09 mm. broad and the narrow part joining it to the mouth has a diameter of 0.044 mm. The nerve ring is 0.166 to 0.17 mm. from the anterior extremity and divides the oesophagus in the ratio of 1 : 3 to 3.6. The tail is extremely short and tapers to a point and measures 0.7 to 0.77 mm. in length. α=9.5–11 ; β=7–8.4 ; γ=7.4–7.7.

Male: It measures 1.6 to 1.9 mm. in length and 0.15 to 0.16 mm. in maximum breadth. The cephalic cuticular inflation measures 0.1 mm. long and the maximum diameter at the level of the inflation is 0.08 mm. The oesophagus measures 0.36 to 0.4 mm. in length. The posterior oesophageal bulb is 0.08 to 0.1 mm. long and has a diameter of 0.07 to 0.078 mm. The anterior bulb has a diameter of 0.04 to 0.056 and the narrow part joining it to the mouth is 0.026 to 0.038 mm. broad. The nerve ring surrounds the oesophagus at a distance of 0.11 to 0.12 mm. from the anterior end and its position divides the oesophagus in the ratio of 1 : 2 to 2.5. The testis reaches far forward and turns posteriorly only at the level of the commencement of the intestine.

The tail is truncated and directed ventrally. There are five pairs of caudal paillae. The anterior and posterior pairs of papillae are large and support the cuticular expansion. The spicule which has a gently bent tip measures, together with the basal expansion, 77 to 81μ in length. The base measures about 12μ across. There is no accessory piece. α=10–12.7 ; β=4–5.3.

Relationships of *E. brevicauda.*

The general similarity of this species to *E. bipapillatus* has been mentioned. It differs from it markedly in the appearance of the female, the relative shortness of the tail (γ=7.4—7.7) in *E. brevicauda* giving it a stumpy appearance. In *E. bipapillatus* the tail is much longer (γ=4.17 to 4.35 in this series and 5 in Cameron's series).

Family: Oxyuridae, Cobbold, 1864.
Species: *Enterobius brevicauda* n.sp.
Host: *Papio porcarius* (Chacma Baboon).
Locality: Southern Rhodesia.
Location: Large intestine.

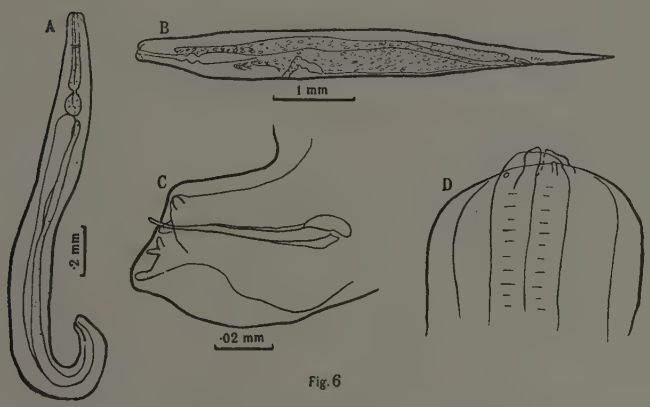

Fig. 6. *Enterobius brevicauda* n.sp.
A, lateral view of male; B, lateral view of female; C, lateral view of posterior end of male; D, ventro-lateral view of head.

Examination of Rhesus Monkey for *Enterobius*.

With the help of Dr. R. E. Rewell of the London Zoological Gardens, the intestines of four monkeys (*Macaca mulatta*) from Calcutta which had died during the air passage to London were examined for helminthic parasites. No *Enterobius* was found but lying free in the small intestines of one specimen was present a large number of *Oesophagostomum* sp.

The examination of the alimentary canals of six stock Rhesus Monkeys which died in the laboratory of the Parasitology Department

of the London School of Hygiene and Tropical Medicine showed no *Enterobius* but large numbers of parasitic adult *Strongyloides* sp. were recovered from the small intestine in every one of them. NIH peri-anal swabs from fourteen Rhesus Monkeys which had been in the laboratory for varying periods were all negative.

Enterobius interlabiata n.sp. from the Feline Douroucouli.

The material consists of two males and several females obtained from the large intestine of a Feline Douroucouli (*Aotus felinus*) from South America which had died in the London Zoological Gardens.

Morphology (Fig. 7).

The cuticle is transversely striated and narrow lateral alae are present. The outline of the lips in side view appears irregular with a thickened cuticle lining the opening into the mouth cavity. The *en-face* view of the head shows three interlabia projecting between the lips. The interlabia are triangular in outline, the ventral one being slightly larger than the others. There are six cephalic papillae which do not appear to be symmetrically arranged in relation to the lips.

Female: The mature specimens measure about 5 mm. in length while the gravid ones are about 6.2 mm. in length. The maximum width varies from 0.29 to 0.33 mm. The vulva is far forward, dividing the body in the ratio of 1 : 2.6 to 3. In the gravid specimens the uteri extend forwards to about the middle of the oesophagus and backwards beyond the anus. The eggs have a smooth shell slightly flattened on one side and measure 52 by 26μ.

The cephalic cuticular inflation measures 0.09 mm. in length and its maximum diameter is 0.11 mm. The oesophagus, which is typically oxyurid in shape, is 0.95 to 0.98 mm. long. The posterior oesophageal bulb is globular and measures 0.11 mm. in diameter. The anterior bulb has a diameter of 0.08 to 0.09 mm., while the narrow part of the oesophagus has a diameter of 0.05 mm. The nerve ring surrounds the oesophagus at a distance of 0.17 to 0.18 mm. from the anterior end, its position dividing the oesophagus in the ratio of 1 : 3. The rectum is prominent and the tail is long and tapering, measuring 1.5 to 1.9 mm. in length. $\alpha = 17.2 - 18.8$; $\beta = 5.3–6.3$; $\gamma = 3.2–3.3$.

Male: The male is 2.1 mm. long and 0.16 mm. in its maximum breadth. The cephalic cuticular inflation measures 0.1 mm. in length and 0.07 mm. in maximum diameter. The oesophagus is 0.57 mm. long. The posterior oesophageal bulb is 0.09 mm. long and has a diameter of 0.07 mm. The anterior bulb has a diameter of 0.05 mm. and the narrow part joining it to the mouth is 0.026 mm. broad. The nerve ring is

0.16 mm. from the anterior end, its position dividing the oesophagus in the ratio of 1 : 2.5. The testis reaches up to 1.2 mm. from the anterior extremity before turning posteriorly.

The tail is truncated and directed ventrally. It has a narrow spike at the end measuring 0.019 mm. in length. There is one large pair of

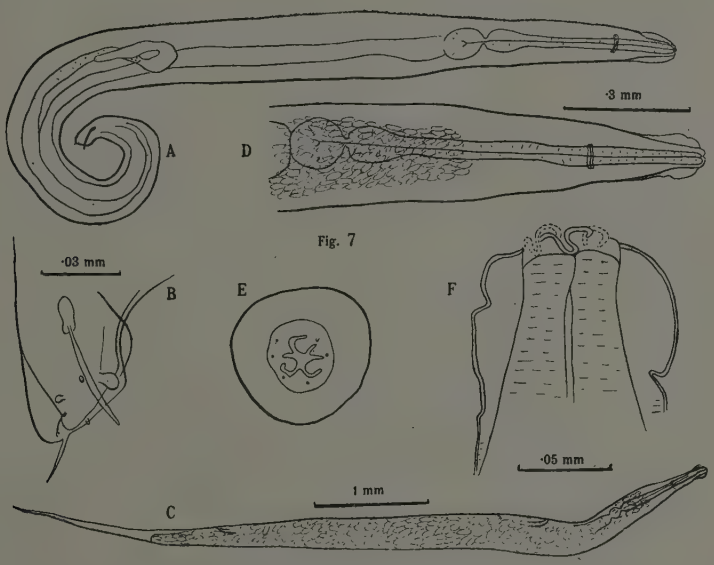

Fig. 7

Fig. 7. *Enterobius interlabiata* n.sp.

A, lateral view of male ; B, lateral view of posterior end of male ; C, lateral view of female ; D, lateral view of anterior end of female ; E, *en-face* view of head ; F, lateral view of head.

precloacal papillae and five smaller post-anal pairs. The spicule, including the broader less chitinised basal portion, measures 0.055 mm. in length. The base which encircles the proximal extremity of the spicule is about 9μ long, by 5μ broad. There is no accessory piece, $\alpha = 18$; $\beta = 3.7$.

Relationships of *E. interlabiata*.

The presence of interlabia serves to distinguish this species from all the other species of *Enterobius*.

Family: Oxyuridae Cobbold, 1864.

Species: *Enterobius interlabiata* n.sp.

Host: *Aotus felinus* (Feline Douroucouli).

Location: Large intestine.

Locality: South America; specimens collected at London Zoological Gardens.

Enterobius callithricis Solomon, 1933, from the Pinché Marmoset.

Several female and two male *Enterobius* were obtained from the caecum of the Pinché Marmoset (*Oedipomidas oedipus*) from South America which had died in the London Zoological Gardens.

The specimens proved to be *E. callithricis* Solomon, 1933, which had been originally described from the marmoset *Hapale* (=*Callithrix*) *jacchus* from South America.

The specimens from the Pinché Marmoset, however, show minor differences. The males are smaller than those of Solomon, measuring only 0.9 and 1.0 mm. in length. The females as well as the males show the fine annular striations of the cuticle.

The Pinché Marmoset (*O. oedipus*) is a new host for *E. callithricis* Solomon, 1933.

Enterobius lemuris Baer, 1935, from the Black Lemur.

Several females of *E. lemuris* Baer, 1935, and another Oxyurid (described below) were obtained from the large intestine of the same Lemur (*Lemur macaco*), from Madagascar, which had died in the London Zoological Gardens.

The specimens of *E. lemuris* could easily be distinguished from the oxyurids present by the anteriorly directed vagina and the much shorter oesophagus.

The specimens of *E. lemuris* are somewhat smaller than those described by Baer from the same host and from the White-fronted Lemur (*Lemur albifrons*).

It measures 4.2 mm. in length and 0.24 mm. in maximum breadth. The cuticular expansion measures 0.1 mm. both in length and maximum diameter. The oesophagus is 0.67 mm. long. The posterior oesphageal

bulb is 0.11 mm. long by 0.09 mm. in diameter. The anterior oesophageal bulb is 0.056 mm. in diameter while the narrow part joining it to the mouth measures 0.037 mm. wide. The nerve ring encircles the oesophagus at a distance of 0.15 mm. from the anterior extremity dividing the oesophagus in the ratio of 1 : 3.5.

The vulva is slightly in front of the midline dividing the body in the ratio of 1 : 1.3. The vagina is long and is directed forwards. The eggs in the uterus measure about 65μ long by 22μ broad. The tail measures 0.62 mm. α=17.7 ; β=6.3 ; γ=6.8.

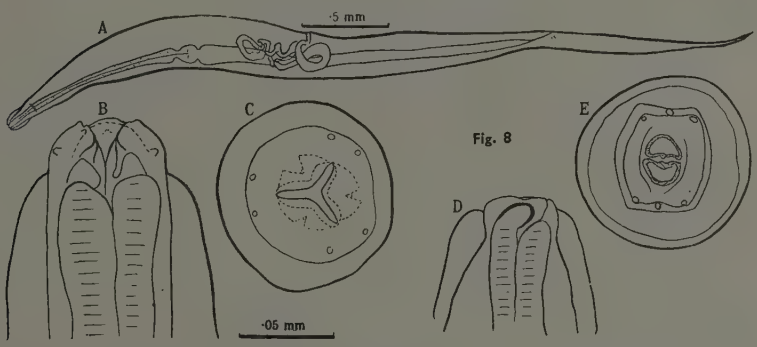

Fig. 8. *Buckleyenterobius dentata* n.sp. (A–C) and *B. lagothricis* (D–E). A, lateral view of female ; B, ventral view of head ; C, *en-face* view of head ; D, lateral view of head ; E, *en-face* view of head.

Buckleyenterobius dentata n.g., n.sp., an Oxyurid from the Black Lemur of Madagascar.

The material consists of several mature, but not gravid, females of a new species of Oxyurid obtained from the large intestine of the Madagascar Black Lemur (*Lemur macaco*) which had died at the London Zoological Gardens. Together with this species in the same animal were several specimens of *Enterobius lemuri* Baer, 1935.

Morphology (Fig. 8, A-C).

The cuticle shows fine annular striations and narrow lateral alae are present. The mouth is surrounded by three lips which project some distance in front of the cuticular expansions. The mouth opens into a small buccal cavity which is provided with three teeth-like cuticular

thickenings projecting from the apices of the three oesophageal sectors. There are six cephalic papillae.

The mature female measures 4.7 to 4.8 mm. in length and 0.3 to 0.33 mm. in maximum width. The cuticular expansion measures 0.13 to 0.14 mm. in length and 0.11 to 0.12 mm. in maximum diameter. The oesophagus is fine and long and measures 1.3 mm. in length. The posterior oesophageal bulb is pyriform in shape being 0.13 to 0.14 mm. in length and 0.12 to 0.13 mm. in maximum diameter. The anterior oesophageal bulb is 0.07 mm. broad and the narrow portion joining it to the mouth has a diameter of 0.05 mm. The nerve ring is far forward being 0.19 mm. from the anterior extremity. Its position divides the oesophagus in the ratio of 1 : 6. The tail is 1.2 mm. long and tapers gradually to a blunt point.

The vulva is situated just in front of the middle of the body dividing it in the ratio of 1 : 1.3 to 1.4. In most of the specimens the vulvar orifice and the neighbouring area of the surface is covered with cement substance indicating that males of this species were probably present but had not been collected. The vagina begins as a short muscular tube running at right angles to the surface, then dilates into a non-muscular tube which forms a loop before it divides into the two uteri in front of the level of the vulvar opening. No eggs were present in any of the specimens. α=14 to 16 ; β=3.6 to 3.7 ; γ=4.

Discussion

Cameron (1929) was the first to describe a Primate Oxyurid with oesophageal teeth. He records *Enterobius atelis* from the Spider Monkeys, *Ateles ater*, *A. grisescens* and *A. paniscus* from South America. Buckley (1931) described two Oxyurid species with oesophageal teeth, *E. lagothricis* and *E. duplicidens*. The species described here is different from these because the females of *E. atelis*, *E. lagothricis* and *E. duplicidens* have only two lips and two oesophageal teeth in each.

Kreis (1932) described an Oxyurid, *Oxyuronema atelophora* from the Spider Monkey (*Ateles geoffroyi*) from Panama. This has a mouth cavity with cuticular skeleton but no teeth. It differs from the above-mentioned oxyurids in the possession of three movable oesophageal spears and in the female having only one ovary. Kreis reports that in this species the anterior oesophageal bulb is longer than broad and very close to the posterior bulb in young mature females (gravid specimens) whereas in the old mature females, the anterior bulb becomes wider than long and moves almost to the middle of the oesphagus. β changes from 5.5 to 6.5 with an average of 6.1 in mature

females to 15.2 to 15.8 with an average of 15.5 in old mature females. These differences seem so great to the writer that the suggestion is made that Kreis was probably dealing with two different species of Oxyurids in the same host. Kreis (1940) describes *Oxyuris armata* from the Sacred Baboon (*Papio hamadryas*) from German East Africa. The female is 29.5 mm. long, has a large cylindrical mouth cavity with two teeth in front and cuticular markings in front of the oesophagus. $\alpha=43.3$; $\beta=9.3$; $\gamma=16.9$. These two oxyurids of Kreis are so different from the ones mentioned above that they need not be considered here.

The species described here as new has more points in common with *E. atelis*, *E. lagothricis* and *E. duplicidens* than with other species of *Enterobius* from Primates and it is proposed to create a new genus for them. It is named *Buckleyenterobius* n.g. after Professor J. J. C. Buckley who described two species belonging to this group and who at the same time suggested that there appeared " to be ample justification for the erection of a new genus for these two species and *E. atelis*." Baer (1935) is also of the same opinion for he says, " Il faut encore y ajouter deux espèces décrites plus récemment par Buckley (1931) chez *Lagothrix humboldtii* Geoffroy. Il semblerait même, d'après Buckley, que le deux espèces décrites par lui constituent les types d'un nouveaux genre dan lequel il faudrait également faire rentrer *E. atelis* Cameron. Ces trois espèces auraient en common la presence dans la bouche de dents chitineuses."

It is doubtful if *E. sceleratus* Travassos, 1925, should be included in this group. The position and size of the two teeth on the ventro-lateral lips suggest that it should not be transferred to this new genus.

The following will therefore constitute the known species of *Buckleyenterobius* n.g. :—

Buckleyenterobius atelis (Cameron, 1929).

B. lagothricis (Buckley, 1931).

B. duplicidens (Buckley, 1931).

B. dentata n.sp.

Generic diagnosis: Oxyurinae : Cuticle of anterior end inflated. Lips distinct or indistinct, two or three. Two or three oesophageal teeth project into the mouth cavity. Narrow lateral alae present. Oesophagus with an anterior and a distinct posterior bulb connected by a narrow neck. Caudal end of male truncate, with alae supported by pedunculate papillae. A terminal spike in male tail present or absent. Spicule single. No gubernaculum. Vulva in the anterior

half of the body. Uterine branches parallel. Adult worms in the large intestine of Primates.

Genotype: *Buckleyenterobius atelis* (Cameron, 1929).
Family: Oxyuridae Cobbold, 1864.
Sub-family: Oxyurinae Hall, 1916.
Genus: *Buckleyenterobius* gen. nov.
Species: *B. dentata* sp. nov.
Host: *Lemur macaco* (Black Lemur).
Location: Large intestine.
Locality: Madagascar (collected at London Zoological Gardens).

Buckleyenterobius atelis (Cameron, 1929) from Spider Monkeys, *B. lagothricis* (Buckley, 1931) and *B. duplicidens* (Buckley, 1931) from Humboldt's Woolly Monkey.

Kreis (1932) commenting on *Oxyuronema atelophora* makes the following statement, " When one examines the living nematodes, one can see that in all specimens the head has the same type of cuticula as the rest of the body. The formations of the anterior end, which are partly given by Cameron (1929, Figs. 24, 25, 17 and 18) and Buckley (1931) are caused by the preservation of the specimens."

In view of the above statement the type and paratype material of *B. atelis* (Cameron, 1929), *B. lagothricis* (Buckley, 1931) and *B. duplicidens* (Buckley, 1931) in the helminthological collection of the London School of Hygiene and Tropical Medicine was carefully re-examined. There was also available for study a more recent collection consisting of many female *B. lagothricis* from *Lagothrix humboldtii*.

The examination of large numbers of specimens has tended to confirm the findings of Cameron and Buckley regarding the formations of the anterior end of *B. atelis*, *B. lagothricis* and *B. duplicidens*.

The accompanying illustrations (Fig. 8, D–E) of the anterior end of the female *B. lagothricis* from the more recent collection (and therefore somewhat differently fixed and preserved from the material from which the type species was described) shows little departure from those of Buckley. The *en-face* view of the head shows what appears to be the relic of the lips. They are wider apart in these specimens and do not seem to quite meet at the sides. It would appear that with the development of the oesophageal teeth in this species the lips have become modified into relatively functionless dermal collars. In the end-on view of the somewhat rectangular head there seems to be four additional minute papillae internal to the corner papillae. The oesophageal teeth in side view are seen to be cuticularized extensions

of the anterior extremities of the ventro-lateral sectors of the oesophagus. They project anteriorly and dorsally overlapping the anterior extremity of the dorsal sector of the oesophagus which is only slightly cuticularized.

Some General Remarks on the species of *Enterobius* Leach.

Cameron (1929) reviewed the species *Enterobius* in Primates and made certain generalisations. He qualified his remarks by pointing out that " many species are inadequately described and many other species of monkeys have to be examined before the series will be sufficiently extensive to justify any results of value to anthropology."

It is more than twenty years since this was written. Since then, several of the known species of *Enterobius* have been redescribed, fourteen new hosts have been added to the list, eleven new species have been described, one species has been transferred from the genus *Oxyuris* to *Enterobius* and three species previously included in the genus *Enterobius* have been transferred to a new genus. This is, therefore, a suitable opportunity to re-examine the whole subject of the species of *Enterobius* and their relationship to their hosts.

Does each Genus of the Primate Host have its own Species of *Enterobius*?

Cameron (1929) first propounded the theory that each genus of the Primates has its own species of *Enterobius*. He considers *E. simiae*, which MacCallum (1921) described from the Orang Utan, a synonym of *E. faecundus*. He dismisses Schneider's (1866) record of *E. minutus* from *Ateles paniscus* as being due to the confusion of two species under one name and the record of *E. bipapillatus* from *Pithecus entellus* by Baylis (1928) as mistaken diagnosis.

Buckley (1931) records what he considers to be a striking exception to this rule when he described *E. lagothricis* and *E. duplicidens* from one Woolly Monkey, *Lagothrix humboldtii*. He qualifies it, however, by remarking, " Again, it may be argued that the species in question do not really belong to the genus *Enterobius*, and hence, the rule is inapplicable to them." These species have, in fact, been transferred to a new genus.

Additional exceptions to the rule are the finding of *E. vermicularis* in four and *E. callithricis* in two genera of Primates and of more than one species of *Enterobius* in the Primate genera *Anthropopithecus*, *Pongo*, *Aotus* and *Oedipomidas*.

The restriction of one species of parasite to one genus of host

would suggest the existence of some degree of host specificity. The finding of *E. vermicularis*, the human parasite in such a wide range of Primates as the chimpanzee, the Lar Gibbon and the Lion Marmoset definitely rules out the existence of any marked host specificity. Buckley (1931) who discusses this problem suggests that the restriction of each species of *Enterobius* to one kind of host is only one of habit and that a physiological host-parasite relationship has not become established. Under artificial conditions such as obtain in a Zoo, the opportunity for one genus of monkey to be exposed to infection with a species of parasite associated with another genus of host would be great and the infection would occur.

It is possible that under natural conditions, when accidental infections would be rare, this generalisation may still be found to be broadly applicable. Re-examination of the type material of species described in the early years may show that some of the recorded double infections are incorrect.

It is no doubt true that the genus *Enterobius* evolved with and parallel to its Primate hosts. It is also probably true that the evolution of the parasite has been slower than that of the host, but in the present state of our knowledge it cannot be said that the parasite has lagged behind to the extent that each species of *Enterobius* is confined to one genus of Primate host only.

At the same time, in spite of the long association between the host and parasite, little host-specificity seems to have been established as evidenced by *E. vermicularis* of man being recovered from the S. American Marmoset, *Leontocebus rosalia* kept in the London Zoological Gardens. The possibility of human infection of *Enterobius* from keeping pet monkeys, etc., cannot be disregarded although it may be argued that man being the most recently evolved of the Primates, his *Enterobius* would have had less time to establish host-specificity than the species of *Enterobius* of other Primate hosts.

The finding of *E. vermicularis* in a wide range of Primate hosts is of great significance to research workers. Several problems relating to the life history of *E. vermicularis* such as internal auto-infection, retrofection, etc., remain to be solved. The possibility of using small Primates such as the Gibbon and Marmoset as experimental animals should hasten the day of elucidation of such problems.

It is necessary for workers recording *Enterobius* infections to state if the host animal had been kept in a zoo and, if possible, for what length of time.

Do the Species of *Enterobius* form a correspondingly related evolutionary Series as their respective Hosts?

Cameron (1929) concludes from his survey of the species of *Enterobius* in Primates that forms most closely related to the human parasite seem to be found in apes, while those in Old World Monkeys seem to be closer to *E. vermicularis* than those in New World Monkeys, but not so close as those in Apes. He does not, unfortunately, single out the morphological data or the criteria on which he bases his conclusions.

An attempt was made to determine this by arranging the species of *Enterobius* in the order of the systematic position of their Primate hosts and recording against each parasite the features generally considered to be of taxonomic value.

It became evident that none of the characters of the parasite shows a gradation in correspondence with the evolutionary position of the host. The most outstanding feature is the presence in the *Enterobius* of the New World Primates of a terminal spike in the males. The only exception, other than finding the spikeless *E. vermicularis* in the S. American Lion Marmoset, is the finding of the spiked *E. nycticebi* in the Far East Loris. Generally speaking, the spicules of the Old World Primates seem to be slightly more complicated with a better development of the basal portion than those of the New World Primates.

That there should be a distinction between the *Enterobius* of the Old World and New World Monkeys is not surprising. These two groups of Primates have been distinct for a long time, since no fossil remains of monkeys intermediate between these have been discovered. While it is probably true that the *Enterobius* of the Cercopithecidae are not so close to *E. vermicularis* as those of the Simiidae, a study of the morphological features of these parasites do not permit of such a conclusion as arrived at by Cameron with any degree of certainty.

The Division of the Genus *Enterobius* into two Subgenera.

It has already been mentioned that the tail spike in the male is present in all the *Enterobius* of Platyrrhine Monkeys and absent in the *Enterobius* of man and all the Catarrhine Monkeys.

South America is the home of several archaic forms of animals and the Platyrrhines stand at the base of a series of monkeys. The spike in the male tail may appear to be a trivial character but further investigation may show that it is homologous with the tail of allied Oxyurids of lower vertebrates.

LeRoux (1930) shows that sexually immature males of *E. polyzoon* have long pointed tails, although in the mature forms the tail is truncated and without a spike. Kreis's (1944) illustration of the sexually immature male of *E. sciuri* shows a distinct tail which in the mature form is replaced by the tail spike. If this is found to be general, one would be justified in considering this an instance of ontogeny recapitulating phylogeny and regarding the tail spike as characterizing the more "primitive" (used in the sense of parasites which have retained something of the structures of their early ancestors) group of *Enterobius*. The tail spike in the male would then be of great significance as a group character.

The presence of the tail spike in a member of the Old World Lorisidae, *E. nycticebi* from Sarawak is not easy to explain. Baylis (1928) had obtained his material from Mt. Poi and there can be no question of accidental infection from a New World Monkey. It is possible that the Slow Loris being an archaic animal isolated in the tropical forests of South-East Asia, has undergone little evolutionary change and with it its parasite.

The absence of the tail spike in the male of *E. lemuris* from the lemurs of Madagascar, where apes and monkeys are not known to occur, is probably due to the *Enterobius* in them having undergone a greater degree of evolutionary change than that in the Slow Loris.

It has been already mentioned that, in general, in the *Enterobius* of Old World Primates, the spicule is somewhat more complex in shape and has a better-developed basal portion. There are probably other differences which have been overlooked.

It would appear, therefore, that there is sufficient justification to regard the *Enterobius* of the New World Primates as belonging to a different subgenus from those of the Old World Primates. Accordingly, the genus *Enterobius* Leach, 1853, is sub-divided into two and those without a spiked tail in the male are included in the subgenus *Enterobius*, and those with, in the subgenus *Trypanoxyuris*. The latter was given as a new generic name by Vevers (1923) for his new species of Oxyurid *T. trypanuris*, which has since been transferred to the genus *Enterobius*.

I. Subgenus : *Enterobius* n.subg.
Type-species : *Enterobius* (*Enterobius*) *vermicularis* (Linnaeus, 1758).

II. Subgenus : *Trypanoxyuris* n.subg.
Type-species : *Enterobius* (*Trypanoxyuris*) *trypanuris* (Vevers, 1923).

A Note on the Species of *Enterobius* in Rodents.

Von Linstow (1909) described *Oxyuris polyzoon* from the Cape Ground Squirrel (*Geosciurus capensis*) from S.W. Africa. LeRoux (1930) redescribed this species which he had collected from the same host in S. Africa and transferred it to the genus *Enterobius* Leach, 1853. The tail of the male of *E. polyzoon* (v. Linstow, 1909) has no spike and therefore belongs to the subgenus *Enterobius*.

Cameron (1932) described *E. sciuri* from the Grey Squirrel (*Sciurus carolinus*) from Scotland. This is a North American squirrel which has been introduced to the British Isles since 1889 and which has firmly established itself in this country. Rausch and Tiner (1948) record *E. sciuri* from Michigan, U.S.A., from the Fox Squirrel (*Sciurus niger rufiventer*) and the Eastern Flying Squirrel (*Glaucomys volans*). The male of *E. sciuri* Cameron, 1932, has a spiked tail and belongs to the sub-genus *Trypanoxyuris*, together with the species of *Enterobius* of the New World whence it was probably introduced to Britain by *Sciurus carolinus*.

Kreis (1944) described as a new species *E. sciuri* from the European Squirrel (*Sciurus vulgaris*) from Berne, Switzerland. The earlier use of the name *E. sciuri* by Cameron has been brought to the notice of Kreis, who says he was unaware of Cameron's work. A comparison of the two descriptions shows that Cameron's and Kreis's species with the same name are in most respects identical. The main difference seems to be in the absence of caudal papillae in the male of Kreis's species. Cameron has described three pairs of caudal papillae, but he states they are small and inconspicuous. It is suggested that *E. sciuri* Kreis, 1944, should be treated as a synonym of *E. sciuri* Cameron, 1932.

The finding of *E. sciuri* with a tail spike in the male in an European Squirrel is interesting. The probable explanation is that *Sciurus vulgaris* has become infected in recent years by *E. sciuri* from *S. carolinus* which, in turn, brought the infection from the New World. There seems to be no record of *S. carolinus* from the European Continent but its successful establishment in the British Isles would strongly suggest that it has already arrived on the mainland of Europe.

There is one record of a female Oxyurid from *Rattus norvegicus* var. *albus* in Japan which Yamaguti (1935) describes as *Enterobius muris* n.sp. He considers that it is closely related to *E. vermicularis* but that the " differences may be of generic importance."

It is interesting to speculate on the occurrence of these essentially Primate Oxyurids in Rodents. They could have arisen as modifications

of the Oxyurids of the ancestral Rodents but, in the absence of species of *Enterobius* in other vertebrates, it seems more likely that the squirrels became infected from Primates in more recent years. The arboreal life led by many squirrels would provide a ready explanation for their acquiring parasites of monkeys.

LeRoux (1930 and 1940) has drawn attention to the fact that the Oesophagostomes of Primates are more closely related to those of Rodents than of the Ruminants. The finding of the closely related Oxyurid *Syphacia obvelata* of rats and mice and of *Hymenolepis* of Rodents in man is also worthy of mention in this connection.

Summary.

1. The value of the hanging-drop technique as used by Professor Buckley for the examination of the different views of the tip of tail and head of small nematodes is stressed. The spicules of various species of *Enterobius* have been examined with polarized light and the significance of the basal portion of the spicules is discussed.

2. *E. vermicularis* is recorded for the first time from the Chimpanzee (*Anthropopithecus troglodytes*), the Lar Gibbon of Malaya (*Hylobates lar*) and the Silky or Lion Marmoset (*Leontocebus rosalia*) all of which had been in the London Zoological Gardens for varying periods.

3. The hitherto unknown male of *E. anthropopitheci* is described and the female is redescribed.

4. A new species, *E. buckleyi* from the Orang Utan (*Pongo pygmaeus*) is described.

5. A new species, *E. lerouxi* from the Gorilla (*Gorilla gorilla*) is described. This is the first record of an *Enterobius* from this host.

6. *E. bipapillatus* is described and recorded for the first time from the Guenon Monkey (*Cercopithecus aethiops*) from S. Rhodesia.

7. A new species, *E. brevicauda* is described from the Chacma Baboon (*Papio porcarius*) from S. Rhodesia. This is the first record of an *Enterobius* from this host.

8. Negative findings for *Enterobius* are recorded from ten post-mortem examinations and fourteen NIH swab examinations of Rhesus Monkeys (*Macaca mulata*).

9. A new species, *E. interlabiata* is described from the Feline Douroucouli (*Aotus felinus*) from S. America. This is the first record of an *Enterobius* from this host.

10. In view of the criticism of Kreis (1932) the type and paratype material of *E. atelis*, *E. lagothricis* and *E. duplicidens* and some more recent collection of *E. lagothricis* have been re-examined and the findings of Cameron (1929) and Buckley (1931) confirmed.

11. *E. callithricis* is recorded for the first time from the Pinché Marmoset (*Oedipomidas oedipus*) from S. America.

12. *E. lemuris* is recorded from the Black Lemur (*Lemur macaco*) from Madagascar and measurements of taxonomic importance are given for comparison with those of Baer (1935).

13. A new genus, *Buckleyenterobius*, containing a new species, *B. dentata*, is described from the Black Lemur (*Lemur macaco*) from Madagascar. *E. atelis*, *E. lagothricis* and *E. duplicidens* are transferred to this new genus.

14. The generalisation of Cameron that a single species of *Enterobius* is confined to a single genus of Primate host is examined in the light of the additional information available.

15. The presence of a tail spike in all the male *Enterobius* of the New World Primates and its absence, with one exception, in the Old World Primates is noted and the genus *Enterobius* Leach, 1853 is split into two subgenera, namely *Enterobius* n.subg. and *Trypanoxyuris* n. subg.

16. Some comments are made on the *Enterobius* spp. of Rodents and *E. sciuri* Kreis, 1944, is sunk as a synonym of *E. sciuri* Cameron, 1932.

17. A list is appended giving the known records of *Enterobius* spp. and *Buckleyenterobius* spp. and their hosts.

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TABLE II.

Record of *Enterobius* spp. from Mammalian Hosts.
Order: PRIMATES.

Host	Locality	Species	Recorded by
ANTHROPOIDEA			
Family: HOMINIDAE			
Homo sapiens (Man)	Cosmopolitan	*E. vermicularis*	
CATARRHINE (OLD WORLD) MONKEYS			
Family: SIMIIDAE (= PONGIDAE)			
Anthropopithecus troglodytes (Chimpanzee)	Tropical Africa	*E. anthropopitheci*	Gedoelst, 1916
		E. anthropopitheci	Sandosham, 1950.
	Tropical Africa (London Zoo)	*E. vermicularis*	Sandosham, 1950.
Pongo pygmaeus (Orang Utan)	Malaysia	*E. faecundus*	v. Linstow, 1879.
	"	? *E. simiae*	MacCallum, 1925.
	"	*E. buckleyi*, n.sp.	Sandosham, 1950.
Gorilla gorilla (Gorilla)	Tropical Africa (London Zoo)	*E. lerouxi*, n.sp.	Sandosham, 1950.
Family: HYLOBATIDAE			
Hylobates lar (Lar Gibbon)	Malaya (London Zoo)	*E. vermicularis*	Sandosham, 1950.
Family: CERCOPITHECIDAE			
Pithecus aygula (Mitred Monkey)	Assam	*E. pitheci*	Cameron, 1929.
Pithecus entellus (Langur)	India	? *E. bipapillatus*	Baylis, 1923.
" Monkey "	Central Africa	*E. bipapillatus*	Gedoelst, 1916.
Cercopithecus sabaeus (Green Monkey)	West Africa	*E. bipapillatus*	Cameron, 1929.
Cercopithecus aethiops (Guenon Monkey)	S. Rhodesia	*E. bipapillatus*	Sandosham, 1950.
Papio porcarius (Chacma Baboon)	S. Rhodesia	*E. brevicauda* n.sp.	Sandosham, 1950.
PLATYRRHINE (NEW WORLD) MONKEYS			
Family: CEBIDAE			
Aotus trivirgatus (Owl-faced Monkey)	S. America	*E. microon*	v. Linstow, 1907.
Aotus felinus (Feline Douroucouli)	S. America (London Zoo)	*E. interlabiata* n.sp.	Sandosham, 1950.
Pithecia monachus (Hairy Saki)	S. America	*E. trypanuris*	Vevers, 1923.
Saimiri sciurea (Squirrel Monkeys)	S. America	*E. sceleratus*	Travassos, 1925.
Saimiri orstedii (Red-backed Squirrel Monkey)	C. America	*E. sceleratus*	Travassos, 1925.
Ateles geoffroyi (Black-haired Spider Monkey)	C. America	*E. trypanuris*	Canavan, 1929.
Ateles paniscus (Red-faced Squirrel Monkey)	S. America	*E. minutus*	Schneider, 1866.
Alouatta seniculus (Red Howling-Monkey)	S. America	*E. minutus*	Schneider, 1866. Theil, 1925.
Alouatta caraya (Black Howling-Monkey)	S. America	*E. minutus*	Schneider, 1866. Travassos, 1925.
Family: HAPALIDAE			
Hapale jacchus (Common Marmoset) (syn. *Callithrix jacchus*)	S. America	*E. callithricis*	Solomon, 1933.
Oedipomidas oedipus (Pinché Marmoset)	S. America (London Zoo)	*E. callithricis*	Sandosham, 1950.
Leontocebus rosalia (Lion Marmoset)	S. America (London Zoo)	*E. vermicularis*	Sandosham, 1950.
LEMUROIDEA			
Family: LEMURIDAE			
Lemur brunneus (Black-headed Lemur)	India	? *E. anthropopitheci*	Baylis and Daubney, 1922.
Lemur albifrons (White-fronted Lemur)	Madagascar	*E. lemuris*	Baer, 1935.
Lemur macaco (Black Lemur)	Madagascar	*E. lemuris*	Baer, 1935. Sandosham, 1950.
Family: LORISIDAE			
Nycticebus borneanus	Sarawak	*E. nycticebi*	Baylis, 1928.
Nycticebus concang (Slow Loris)	Malaya	*E. nycticebi*	Cameron, 1929.

TABLE II (*contd.*).

Order : RODENTIA.

Family : SCIURIDAE			
Geosciurus capensis (Cape Ground-Squirrel)	S. and S.W. Africa	*E. polyzoon*	v. Linstow, 1909. LeRoux, 1930.
Sciurus carolinus (Grey Squirrel)	N. America and introduced to England	*E. sciuri* Cameron, 1932	Cameron, 1932.
Sciurus niger rufiventer (Fox Squirrel)	N. America	*E. sciuri* Cameron, 1932	Rausch and Tiner, 1948.
Glaucomys volans (Eastern Flying Squirrel)	N. America	*E. sciuri* Cameron, 1932	Rausch and Tiner, 1948.
Sciurus vulgaris (European Squirrel)	Europe	? *E. sciuri* Kreis, 1944	Kreis, 1944.
Family : MURIDAE			
Rattus norvegicus v. albus	Japan	*E. muris*	Yamaguti, 1935.

TABLE III.

Record of *Buckleyenterobius* spp. from Mammalian Hosts.
Order : PRIMATES.

Host	Locality	Species	Recorded by
ANTHROPOIDEA			
PLATYRRHINE (NEW WORLD) MONKEYS			
Family : CEBIDAE			
Ateles ater (Black-faced Spider-Monkey)	S. America	*B. atelis* (Cameron, 1929)	Cameron, 1929.
Ateles grisescens (Grizzled Spider-Monkey)	S. America	*B. atelis* (Cameron, 1929)	Cameron, 1929.
Ateles paniscus (Red-faced Spider-Monkey)	S. America	*B. atelis* (Cameron, 1929)	Cameron, 1929.
Lagothrix humboldtii (Woolly Monkey)	S. America	*B. lagothricis* (Buckley, 1931)	Buckley, 1931. Sandosham, 1950.
Lagothrix humboldtii	S. America	*B. duplicidens* (Buckley, 1931)	Buckley, 1931.
LEMUROIDEA			
Family : LEMURIDAE			
Lemur macaco (Black Lemur)	Madagascar	*B. dentata* n.sp.	Sandosham, 1950.

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Index to Volume XXIV

PAGE

Anthropopithecus troglodytes, new host of *E. vermicularis* ... 172
Aotus felinus, host of *E. interlabiata*, n.sp. 188
Barium antimonyl tartrate in the treatment of Gapeworm Disease 53
Buckleyenterobius atelis, n. comb. 193
dentata, n.g., n.sp., generic diagnosis ... 193
in Black Lemur 191
morphology 191
duplicidens, n. comb. 193
lagothricis, n. comb. 193
Cattle host of *Paramphistomum hiberniae*, n. sp. 158
scotiae, n. sp. 162
Cercopithecus aethiops, new host of *E. bipapillatus* 184
Chlorphenol, phytocidal action 96
tested against *Anguillulina dipsaci* 93
Aphelenchoides ritzema-bosi ... 93
Clover seed 94
Heterodera rostochiensis 97
Teazle seed 94
Control of poultry parasites by Methyl bromide 138
Dictyocotyle coeliaca, anatomy 16
incidence in Skates and Rays 15
systematic position... 18
Diffusates, potato-root, influencing eelworm emergence ... 86
D–D mixture tested against *Heterodera marioni* 123
Enterobius anthropopitheci, female redescribed 174
male described 174
bipapillatus, in Guenon Monkey, new host from S. Rhodesia 184
redescribed 184
brevicauda, n.sp., in Chacma Baboon from S. Rhodesia 186
morphology 186

PAGE

buckleyi, n.sp., in Orang Utan from Borneo... ... 178
morphology 178
callithricis, in Pinché Marmoset, new host from S. America 190
interlabiata, n.sp., in Feline Douroucouli from S. America 188
morphology 188
lemuris, in Black Lemur, described 190
lerouxi, n.sp., in Gorilla from Africa 181
morphology 181
new subgenus of *Enterobius* 198
sciuri Kreis, 1944, synonym of *E. sciuri* Cameron, 1932 199
spp. in Rodents discussed 199
vermicularis in Chimpanzee 172
Lar Gibbon, from Malaya 173
Lion Marmoset, from Brazil ... 173
Man 171
Fasciola hepatica, infective to *Limnaea glabra* 67
palustris 67
stagnalis 65
truncatula 63
non-infective to *Limnaea auricularia* ... 68
partly infective to *Limnaea pereger* 67
pathogenic to *Limnaea* spp. 69
Gametogenesis in *Gigantocotyle bathycotyle* 1
Gapeworm Disease, prevention with Phenothiazine 54
treatment with Barium antimonyl tartrate 53
Gigantocotyle bathycotyle, gametogenesis 1
genitalia 2
oogenesis 9
spermatogenesis 4
Gorilla gorilla, host of *E. lerouxi*, n.sp. 181
Hatching rate analysis in cysts of *Heterodera rostochiensis* ... 75

PAGE

Heterodera rostochiensis, cysts, effect of different diffusates on emergence 86
form of hatching curve... ... 75
rate of larval emergence ... 75
Hylobates lar, new host of *E. vermicularis* 173
Iodine solutions tested against eelworm infected seeds ... 92
eelworms 91
Keratitis in pheasants due to Phenothiazine 61
Larus canus, new host of *Syngamus trachea* 42
Lemur macaco, host of *Buckleyenterobius dentata*, n.g., n.sp. ... 191
Leontocebus rosalia, new host of *E. vermicularis* 173
Limnaea spp., susceptibility to *Fasciola hepatica* in Britain ... 63
Melanocorypha calandra, new host of *Syngamus trachea* ... 41
Methyl bromide lethal to helminth vectors in soil 142
nematode eggs in soil 142
Nematocides and plant nematodes 91, 93, 123
New Trichostrongylids in Northern Rhodesia 23, 28
Oedipomidas oedipus, new host of *E. callithricis*... 190
Ourebia ourebi, host of *Paracooperia mazabukae*, n.sp. 28
Papio porcarius, host of *E. brevicauda*, n.sp. 186
Paracooperia mazabukae, n.sp., in Northern Rhodesia 28
Oribi 28
morphology 29
Paramphistomum hiberniae, n.sp., from cattle in Ireland ... 158
Netherlands ... 158
Scotland ... 158
morphology ... 158
scotiae, n.sp., from cattle in Ireland 162
Scotland... ... 162
morphology ... 163
Phenothiazine, causing Keratitis in pheasants 61
controlling Gapeworm Disease 54
Plant nematodes treated with Chlorphenol 93
Iodine 93
Pongo pygmaeus, host of *E. buckleyi*, n.sp. 179

PAGE

Poultry parasites, eggs destroyed by Methyl bromide in soil ... 137
Raia spp. infected with *Dictyocotyle coeliaca* 15
Rhesus monkeys examined for *Enterobius* 187
Rodent hosts of *Enterobius* spp. discussed 199
Root-Knot Eelworm treated with D–D mixture 123
Scottish hill sheep, seasonal variation in egg output 101
Soil sterilization by Methyl bromide 137
Stem eelworm in weeds in agricultural crops 145
horticultural crops 148
transferred from clover to onion 151
oats to strawberry 150
strawberry to onion 151
teazle to onion 151
Syngamus gracilis, synonym of *S. trachea* 35
merulae, ,, ,, ,, ... 36
parvus, ,, ,, ,, ... 36
trachea, new record in *Larus canus* 42
Melanocorypha calandra ... 41
revised host list 41, 42
synonymy 38, 39
Taurotragus oryx, host of *Trichostrongylus leiperi*, n.sp. ... 23
Technique for counting trematode eggs 47
Tomatoes and soil treatment with D–D mixture 123
Transference, experimental, of *Ditylenchus dipsaci* between crop plants 150
Trematode eggs, counted by new technique 47
Trichostrongylus leiperi, n.sp., in Eland 23
Northern Rhodesia 23
morphology 24
Trypanoxyuris, new subgenus of *Enterobius* 198
Weed hosts of *Ditylenchus dipsaci* in agricultural crops ... 145
horticultural crops ... 148
Worm egg output, seasonal variation in sheep 101

Index of Authors

	PAGE
CLAPHAM, P. A.	53, 61, 137
EDWARDS, E. E.	123
FENWICK, D. W.	75, 86
HUNTER, G. C. and KILLE, R. A.	15
KENDALL, S. B.	63
LEROUX, P. L.	23, 28
MADSEN, H.	33
MORGAN, D. O., PARNELL, I. W. and RAYSKI, C.	101
SANDOSHAM, A. A.	171
SOUTHEY, J. F. and STANILAND, L. N.	145
STANILAND, L. N.	91
WHITLOCK, H. V.	47
WILLMOTT, S.	1, 155

New Names in Volume XXIV

	PAGE
NEW GENERA.	
BUCKLEYENTEROBIUS Sandosham, 1950	193
NEW SUBGENERA.	
ENTEROBIUS Sandosham, 1950	200
TRYPANOXYURIS Sandosham, 1950	200
NEW SPECIES.	
BUCKLEYENTEROBIUS DENTATA Sandosham, 1950 ...	193
ENTEROBIUS BREVICAUDA Sandosham, 1950	186
ENTEROBIUS BUCKLEYI Sandosham, 1950	178
ENTEROBIUS INTERLABIATA Sandosham, 1950 ...	188
ENTEROBIUS LEROUXI Sandosham, 1950	181
PARACOOPERIA MAZABUKAE LeRoux, 1950	28
PARAMPHISTOMUM HIBERNIAE Willmott, 1950 ...	158
PARAMPHISTOMUM SCOTIAE Willmott, 1950	162
TRICHOSTRONGYLUS LEIPERI LeRoux, 1950	23

NEW COMBINATIONS. PAGE

BUCKLEYENTEROBIUS ATELIS (Cameron, 1929) ... 193
BUCKLEYENTEROBIUS DUPLICIDENS (Buckley, 1931) 193
BUCKLEYENTEROBIUS LAGOTHRICIS (Buckley, 1931) 193